THE UNIVERSITY OF ALBERTA

ESTERIFICATION AND ANALYSIS OF SATURATED FATTY ACIDS BY REACTION-GAS CHROMATOGRAPHY

by

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ESTERIFICATION AND ANALYSIS OF SATURATED FATTY ACIDS BY REACTION-GAS CHROMATOGRAPHY

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ABSTRACT

The application of reaction-gas chromatography as an analytical tool in the esterification and determination of saturated fatty acids was examined. An attempt was made to accomplish the esterification and quantitative determination without a previous esterification step outside the reaction-GC instrument as done in conventional methods.

Boron trifluoride-methanol did not prove to be a satisfactory reagent whereas distilled diazomethane in ethyl ether was found to lend itself well to the reaction-GC techniques. Octanoic acid was successfully esterified by diazomethane with yields close to the theoretical. Higher molecular weight acids were not completely esterified using the same method. The residence time of the reaction mixture in the reactor was not found to exert significant influence on the esterification yields.

The reactor and GC system used are described in detail.

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LIST OF SYMBOLS

F_s	response factor of thermal conductivity detector for esters
W_i	weight of internal standard in calibration solution
W_s	weight of ester added to standard solution
A_s	area of ester obtained by electronic integration, arbitrary counts, for calculation of F_s
A_i	area of internal standard obtained by electronic integration, arbitrary counts, for calculation of F_s
$W_{s, th}$	theoretical weight of ester

ESTERIFICATION AND ANALYSIS OF SATURATED FATTY ACIDS BY REACTION-GAS CHROMATOGRAPHY

1. INTRODUCTORY STATEMENT OF THE PROBLEM

1.01 INTRODUCTION

The importance of the analytical methods for the determination of fatty acids stems from the fact that these materials are components of many natural and industrial products. In the form of their esters, they occur in fats, waxes, resins, oils of natural origin and in plasticizers and plastics.

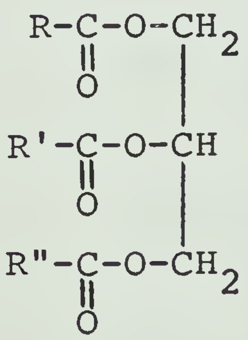
Their importance is mainly attributed to their being integral parts of lipids which are widely distributed in biological systems. A general classication of lipids is[91]: (A) simple lipids, (a) fats, (b) waxes; (B) compound lipids, (a) phospholipids, (b) glycolipids, (c) lipoproteins. A common characteristic of the two major groups is the presence of a fatty acid in ester or amide linkage which can be released by hydrolysis of the lipids. The fatty acids themselves may be divided into (a) saturated; (b) unsaturated; (c) branched chain; (d) cyclic acids. All groups have some characteristics in common, the most interesting being that the fatty acids are mainly monocarboxylic acids with hydrocarbon groups attached to the carboxyl group, as in the general formula R-COOH; and also that virtually all the

acids of the naturally occurring fats have an even number of carbon atoms.

The most abundant saturated fatty acid present in animal fats is palmitic acid (C_{16}) followed by stearic (C_{18}) acid. Other acids of shorter chain (C_{12} to C_{14}) also occur in less abundance as do longer chain acids. Among the unsaturated fatty acids, the monounsaturated have the general formula

$$R\text{-}CH = CH\text{-}(CH_2)_7\text{-}COOH$$

where the double bond first occurs between carbons 9 and 10. Polyunsaturated acids also occur in which R contains double bonds. The cis- form is the configuration found in nature. Thus, oleic acid (cis-9-octadecenoic) and palmitoleic (cis-9-hexadecenoic) are the most abundant unsaturated acids found in animal lipids. The fats, or triglycerides, are known as simple lipids; they are esters of glycerol and fatty acids. Their general formula is



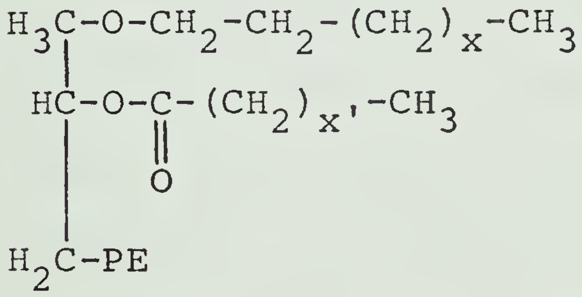
Where R-C=O; R'-C=O; R"-C=O represent fatty acid residues, which can be from either the same or different acids.

and they are the most abundant lipids in nature, consti-

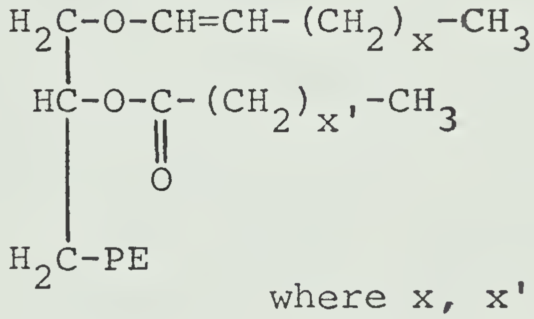
tuting the fats and oils; they are the "fats" in the common sense of the word[92]. In the normal mammal 10% or more of the body weight may be in the form of triglycerides. The waxes are esters of long chain alcohols and fatty acids, instead of glycerol:

$$H_3C-(CH_2)_x-CH_2-O-\underset{\underset{O}{\|}}{C}-(CH_2)_{x'}-CH_3$$

Compound lipids contain additional elements such as S, P or N besides the C, H, O found in simple lipids. Those lipids containing phosphorus are known as phospholipids, in which one of the hydroxyl groups of the glycerol molecule is substituted for phosphoryl choline, phosphoryl ethanolamine or phosphoryl serine. Ethanolamine phospholipids exist in three forms:

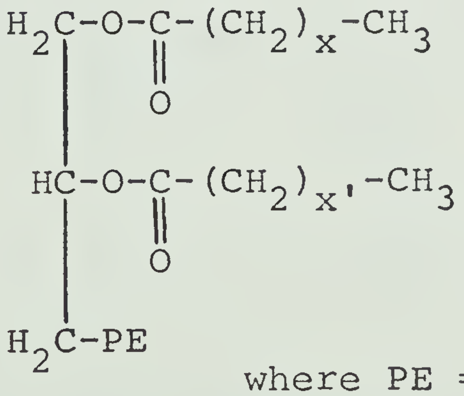


Ether-ester phosphatide



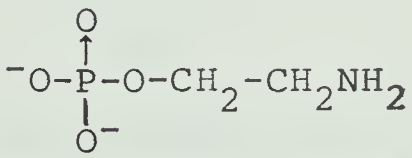
where x, x', ... 8

Vinyl ether-ester phosphatide (plasmalogen)



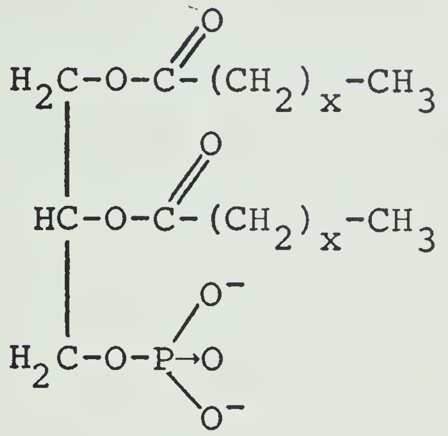
Diester phosphatide (cephalin)

where PE =



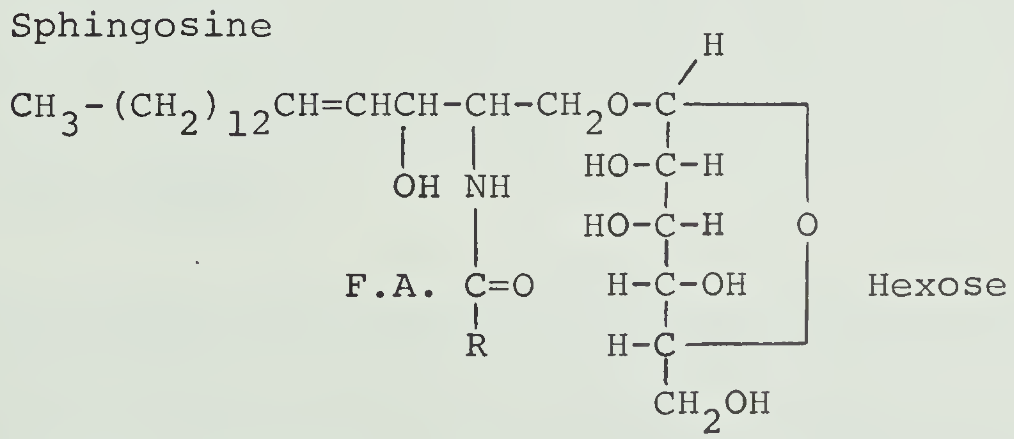
(Phosphoryl ethanolamine)

Choline and serine phospholipids occur also in these forms. The phosphatidic acids are phospholipids which, unlike the lyso compounds, do not contain free bases:



α-Phosphatidic acid

Another group of phospholipids, the sphingolipids or sphingomyelins are composed of a polyvalent aminoalcohol (i.e., sphingosine), phosphoryl choline and a fatty acid. Glycolipids such as cerebrosides and gangliosides contain a sugar as an integral part of the molecule, do not contain phosphorus. On hydrolysis they yield a sugar, frequently a hexose, a nitrogen-containing fragment and a fatty acid:



Other lipids are sulpholipids, the structures of which are

not yet elucidated, and lipoproteins in which stearic, palmitic and oleic acids are the main fatty acid components.

This large variety of lipids perform two main functions in a living organism: one is acting as a fuel reserve for metabolism, the other, they form biological membranes and interfaces where they serve as an intermediate between a water-soluble and an insoluble phase. In the form of waxes, they can function as energy reserves in both plants and animals although more often they appear to serve as a protective cover on the surface of leaves, stems and fruits, and also provide protection for wool fibers and the skin of most fur-bearing animals.

The analytical importance of the fatty acids, present as building blocks in these compounds is then not to be underestimated as is evidenced by the large body of literature in the form of books and research papers dealing with their determination. Since they are usually found both in natural products and industrial materials either bound in larger molecules and in the form of mixtures of varying degrees of complexity, analytical separation is frequently an essential step preceding their identification or determination in a sample.

1.02 DETERMINATION OF ACIDS AND ESTERS BY CHEMICAL METHODS

In this section some representative methods for the determination of the carboxyl and ester functions will be

briefly reviewed.[34] By chemical methods we mean those methods based on chemical reactions involving the carboxyl or ester function as contrasted to physical methods or analytical methods which are not based on chemical reactions. They are also called 'wet methods' and are generally classified as micro, when the procedures require about 0.1 meq; or macro, when they require 1 meq or more of the compound for analysis.

A. Determination of the Carboxyl Function by Neutralization.

If the pK_a values for acids in general are examined, it will be found that there are roughly four categories of acids, with the strong inorganic acids having low pK_a's or perhaps even negative values. The next category are the organic acids with an acid strength close to those of the inorganic and includes the sulfonic, polynitro and polyhalogenated organic acids. The common carboxylic acids such as acetic and valeric are generally considered weak acids with their pK_a's ranging from 3.5 to 5. The "very weak" or last group comprises those acids with pK_a's near 10 such as the phenols and naphthols. The Table 1.01[(34)] lists some acids and their pK_a's for comparison purposes.

The titration methods for neutralization can be carried out in aqueous or nonaqueous solutions, the latter having the larger scope and usefulness. Aqueous titration of acids is limited to the so-called inorganic acids and the organic acids that are soluble in water and have pK_a's as

TABLE 1.01 DISSOCIATION CONSTANTS OF SOME ACIDS

Acid	pK_a	Percent titrated to phenolphthalein end point
Hydrogen bromide	-9	100%
Perchloric	-8	
Sulfuric	0.4	
(HSO_4^-)	1.92	
Trichloroacetic	0.66	100%
Benzenesulfonic	0.70	
Trinitrophenol	0.71	
Dichloroacetic	1.25	
Chloroacetic	2.87	
Bromoacetic	2.90	
Formic	3.75	100%
Benzoic	4.18	
Acetic	4.76	
Valeric	4.86	

low as 8. The titrant normally employed is aqueous NaOH with phenolphthalein as the visual indicator. The slight interference from atmospheric CO_2 should be minimized. The reaction

$$RCOOH + OH^- \rightleftharpoons RCOO^- + H_2O \qquad (1.01)$$

is reversible and dilute ethanol is used as a solvent in order to suppress the reverse reaction. It is used in 50% or 95% solutions either pure or mixed with acetone or dioxan. For nonaqueous titrations sodium methoxide or ethoxide and potassium methoxide solutions are used. Acetone, DMF and benzene-methanol, benzene-isopropyl alcohol as well as others like pyridine can be used as solvents,

the latter for instance being useful in the accurate titration of phenols. The end-point may be detected by means of visual indicators but potentiometric titrations are necessary, especially when the pK_a of the compound sought is not known. Carbon dioxide must be excluded because of the higher sensitivity of alkoxides to it. The temperature is sometimes raised as when a determination is made of higher fatty acids at 65°C using 0.05 N sodium ethoxide.

B. Determination of the Carboxyl Function as Active Hydrogen.

The carboxyl proton reacts with Grignard reagents such as methyl magnesium iodide to liberate methane which is then determined gasometrically in the 0.1 meq range.

$$RCOOH + CH_3MgI \longrightarrow CH_4 + RCOOMgI \qquad (1.02)$$

C. Other Methods for the Determination of the Carboxyl Function.

(a) Decarboxylation,

$$RCOOH \longrightarrow RH + CO_2 \qquad (1.03)$$

is not quantitative for most carboxylic acids. The CO_2 liberated may be determined by titrimetry, or manometrically or, in the 0.1 meq range, by gas chromatography; (b) esterification with methanol-BF_3, the resulting water being deter-

mined by a Karl Fischer titration; (c) gravimetric determination of certain carboxylic acids by precipitation of their metal salts.

D. Determination of the Ester Function.

This is commonly made by the classical saponification method, introduced by Kottstorfer[2] and consists of reacting the ester with a known amount of KOH solution (Eq. 1.04):

$$RCOOR' + KOH \longrightarrow RCOOK + R'OH \qquad (1.04)$$

After heating for a specified period of time the residual alkali is determined by titration with standard inorganic acids. There are several modifications of this titration procedure for adaptation to macro or micro amounts of samples. There are interferences from some other functional groups such as aldehydes and peroxides. Aldehydes show particularly severe interference because of self-condensation reactions which consume caustic. The method is not applicable to esters which exhibit a high degree of steric hindrance.

E. Other methods are

(a) the use of a Grignard reagent; (b) use of lithium aluminum hydride; (c) measurement of the akoxyl content of esters derived from low molecular weight alcohols

by cleavage of the ester group by hydrogen iodide:

$$RCOOR' + HI \longrightarrow RCOOH + R'I \qquad (1.05)$$

Sometimes resort has to be made to gas chromatography for the determination of the alkyl iodide if the parent alcohol has three or more carbon atoms. This and miscellaneous methods are well described in the literature[3,33,34]. Lee[4] discusses the analysis of mixtures by methods based on competing reactions.

1.03 DETERMINATION OF ESTERS AND ACIDS BY SPECTROSCOPIC TECHNIQUES

Acids and esters have been determined by spectrophotometric methods using (a) ferric hydroxamate solutions, the colored species from aliphatic esters absorbing in the region of 530 mμ, those from aromatic esters absorbing around 550-560 mμ. Acids have to be esterified prior to this determination. (b) pinacyanol, a water soluble cationic dye, suggested by Mukerjee[40] for the determination of ppm concentrations of carboxylic acid salts (soaps). The dye and the carboxylate anion react at pH 9.0 to form a colored complex which can be extracted into an organic solvent. The lower molecular weight acids like butyric, hexanoic, do not respond but higher fatty acids give high levels of molar absorptivity such as $1.7\text{-}2.2 \times 10^4$ for

decanoic, 3.5-5.0x10^{4} for lauric acid.

Other spectroscopic techniques. UV and visible. The carboxyl and ester functions do not absorb in those regions of the electromagnetic spectrum. If their determination is to be made, e.g., in the visible region, they must be converted into colored species by reaction with appropriate compounds such as conversion to ferric hydroxamates and others, as discussed above. IR techniques are not used for quantitative determinations without known samples of the authentic compounds. NMR (proton) spectroscopy gives a carboxyl proton resonance at a very low field. Since the carboxyl proton can be determined easily by simple procedures, its determination is rarely if ever made by NMR (proton) spectroscopy, which is mainly used for detection purposes and studies concerned with proton exchange. As to mass spectroscopy, the situation is different because the fragmentation pattern of the carboxyl function has been thoroughly investigated and its identification presents no special problem[41,42]. The same is true of methyl and ethyl esters.

1.04 TECHNIQUES FOR THE SEPARATION OF MIXTURES OF FATTY ACIDS OR THEIR ESTERS

As was mentioned in Section 1.01 in a great majority of cases it is necessary to effect a separation prior to the

determination of acids or esters. Since most of the natural fats and waxes are composed of complex mixtures of glyceride or monoester fatty acids, the quantitative separation of the esters is difficult and often impossible even with the most refined techniques. Their conversion to a single type of monoester such as the methyl ester or to free acids often precedes the separation step proper.

Prior to 1935 the methods of separation used were the now classical ones of crystallization of various metallic salts from a limited number of solvents and distillation of acids or esters with crude apparatus and inefficient fractionating columns.

Adding to the complexity of the problem is the necessity of either hydrolyzing the fat or wax by one of several methods or of transesterifying the natural lipid to produce esters in a single step, depending on whether it is desired to apply a process of separation to the mixture of acids or to the mixture of monoesters. In the case of hydrolysis it will sometimes be necessary to separate the monoacidic constituents and, if an alkaline hydrolysis has been made, liberate the acids from the mixture of soaps formed. Hydrolysis can be carried out by (a) methods using alkali hydroxides or alkoxides; (b) acid hydrolysis methods; (c) enzymatic methods involving lipolytic enzymes; (d) autoclaving methods with or without a catalyst. Esterification methods will be treated in a later section.

Once the desired form for separation has been achieved, distillation or crystallization can now be applied. The early distillations made demanded a prior fractionation of natural mixtures of fatty acids into groups such as (a) volatile from less volatile acids (by steam distillation); (b) saturated from unsaturated acids; (c) separation of the components of the unsaturated fraction from each other; (d) separation of the saturated fraction components from each other. With the evolution of distillation as an analytical technique, by means of more refined instrumentation and the introduction of molecular distillation, a variety of separations was made which contributed to the knowledge of the composition of a variety of natural as well as synthetic fats, oils and waxes. The golden period of application of distillation methods started about the year 1911 when T. P. Hilditch began his researches on the composition of fats of both animal and vegetable origin and continued through 1940, when "precise analytical ester fractionation was universally adopted for the determination of the fatty acid composition of natural lipids and was accompanied by the introduction of new types of highly efficient distillation apparatus, improved techniques of distillation and new objectives of ester fractionation"[35].

The salt-solubility methods are based on the solubilities of salts of saturated and unsaturated fatty acids with metallic ions in water and organic solvents. The solu-

bilities vary with the nature of the metallic ion, chain length, degree of unsaturation and other factors and have been investigated for most of the salts of the alkali, alkaline-earth and heavy metals (Pb, Zn, Fe, Co, Ni, Au, Ag, Hg) in several organic solvents such as benzene, toluene, methanol, ethanol, acetone, diethyl ether, amyl alcohol and mixtures of these and other solvents. The solubility of any one salt in a particular mixture, however, is affected by the relative proportions of all other soluble salts present, by temperature and hence none of the methods devised can be strictly quantitative when applied to complex mixtures of fatty acids obtained from natural products. Among the methods used, the lead salt-diethyl ether method, introduced by Gusserov in 1828 is the oldest and most widely used and one of its modifications is still listed as an official method by the AOCS[38]. Other procedures cited in the literature are (a) the lithium salt-acetone; (b) the barium salt-benzene; (c) the magnesium salt-alcohol methods[35].

As to low temperature crystallization, it was introduced around 1930, the time that the so-called precise fractional distillation was being developed. The technique attempts to separate fatty acids or their esters from solvents at subzero (°C) temperatures. It has been used to separate (a) saturated from unsaturated acids; (b) saturated acids; (c) mono-,di-,tri-, and tetraenoic acids; (d) branched chain saturated acids; (e) glycerides. There

is no standard technique or apparatus for low temperature crystallization and the methods used are entirely empirical, depending on prior knowledge of the composition of the mixtures to which they are to be applied. Several critical factors come into play and they are not easy to control. The latter is one of the causes of the difficulty in giving low temperature crystallization a theoretical treatment, but it has nevertheless been applied for more than fifty years especially for the separation of large amounts of mixtures of acids into predominantly saturated and unsaturated fractions. As an analytical tool it has been applied only to a limited degree.

Countercurrent distribution techniques have been applied to fatty acids, their methyl esters, and triglycerides, as described by Scholfield[35,39]. As will be seen shortly, fatty acid methyl esters may be analyzed more rapidly and efficiently by gas chromatography. For the isolation of volatile esters or fatty acids in larger amounts, however, countercurrent distribution is frequently the best technique available, and for nonvolatile mixtures (e.g., triglycerides), it is sometimes the only satisfactory method for analytical and preparative purposes. Since it employs extremely mild conditions it may be used for the investigation of unstable compounds. It is non-destructive allowing recovery of practically all of the material processed. As contrasted to chromatography and continuous

extraction, the countercurrent distribution process is amenable to mathematical treatment for predicting the behavior of compounds and the attainable degree of separation of mixtures.

The formation of crystalline inclusion compounds (or adducts or complexes) between urea and straight chain or certain branched chain and cyclic compounds is a relatively newer technique in organic chemistry and has found wide application in the fields of fats and petroleum. The complexes can be used to separate acids, esters, alcohols and other derivatives from each other or from fats and other non-complexing substances. This technique as applied to the fatty acid field is described by Swern[35].

Of much more importance and wider application than any of the previously described techniques is liquid chromatography. Its various forms of column, paper and thin-layer chromatography have all been applied to the field of fatty acids and lipid analysis. Column chromatography is applicable to large sample sizes, from 0.1 g to several grams and serves mainly for preparative purposes, although its ultimate goal may be analytical. Classes of compounds such as fatty acids, mono-, di-, and triglycerides can be fractionated from each other or the components of one class can be separated, for example, from a mixture of fatty acids. Paper chromatography is used for smaller samples in the range of 1-100 μg and

mainly for the separation of components of a given chemical class. Paper chromatography of a mixture of fatty acids may require up to 20 hours for development. Thin-layer methods are applicable to samples in the ranges of micrograms to 1 mg, which places it between paper and column chromatography with regard to sample size. It is also a much better way of recovering separated lipids than from paper or glass-paper chromatograms in addition to requiring sometimes only a few hours for the development of a chromatogram of fatty acids. Since the absorbent is in the form of a fine powder layer, fractionation is improved, being sharper than on paper chromatograms. Its major use, however, has been for the separation of lipids into classes; separation of homologous fatty acids and related subfractionation have not yet been investigated as thoroughly by thin-layer chromatography as by the other techniques of liquid chromatography. Schlenk[35] compares the characteristics of liquid chromatography with other methods and lists the following conclusions:

"1. The experimental conditions are milder in liquid than in gas chromatography and distillation; nonvolatile compounds which are not amenable to the latter methods can be separated.
2. Separations may be on a gram, milligram, and microgram scale; they are more specific than those achieved by distillation or crystallization, but not as specific as those attainable by gas chromatography; they are more nearly comparable to those attained by countercurrent distribution.

3. Open chromatograms leave all components of a sample accessible for detection so that, in principle, total analyses can be made.

4. Liquid chromatography is used for preparative rather than analytical separations, particularly when the sample can be analyzed by gas chromatography; it is the most important method for the separation of non-volatile lipids.

5. Liquid chromatography is most useful when combined with other procedures, such as other methods of separation or chemical reactions prior to the application of liquid chromatography.

Of all the methods of separation cited, however, none has had such impact and explosive development as gas chromatography. It is well known that the first experiments destined to give birth to gas chromatography, performed by James and Martin[1] were attempts to separate fatty acids from formic through dodecanoic. Woodford[35], describing the technique as applied to the fatty acid field, states:

"The study of fatty acids has received considerable impetus from the application of the powerful technique of gas-liquid chromatography(GLC). Other methods for the analysis of fatty acid mixtures, such as fractional distillation, low-temperature crystallization, countercurrent distribution, and reversed phase chromatography are still used but in many instances they have been superseded by the newer technique because of its speed and accuracy, and especially because a complete quantitative analysis can be carried out with microscopic quantities of material (of the order of 1 mg to 1 microgram). The ability to analyze such minute quantities makes gas chromatography the only method which can be applied in many biochemical problems when the sample size is unavoidably minute."

Although, as Schlenk states[35], liquid chromatography is still the method of choice when nonvolatile compounds are being analyzed, the experimental conditions being milder in LC than GLC, gas chromatography deserves special consideration among the separation techniques. The next section will be devoted to an examination of GLC techniques for the analysis of fatty acids and their derivatives.

1.05 GAS-CHROMATOGRAPHIC ANALYSIS OF FATTY ACIDS AND DERIVATIVES

The analysis of fatty acids is by no means an easy matter. A. T. James[43] states that *"Apart from the petroleum hydrocarbons the fatty acids represent perhaps the most complex group of naturally occurring substances."* Nevertheless, since their separation is of great interest to biochemists and workers in other fields, the efforts made in the direction of analyzing more and more complex mixtures have been enormous and culminated with the paper published by James and Martin[1] where the technique of gas-liquid chromatography was not only described for the first time but also applied to the separation of several fatty acids as already mentioned.

There are several different ways of approaching the problem of fatty acid analysis, depending both on the actual composition of the sample and the techniques one

would like to use.

Concerning the sample it is necessary to know if (a) the fatty acids are in the free state in the sample; or (b) they are present as components of lipids of the various origins already mentioned (phospholipids, glycolipids, etc.) in which case the lipids will have to be decomposed in order to liberate the acids. Once that is known, the decision remains to be made if the fatty acids in both forms of sample are to be determined as free acids or if they are to be determined as some of their derivatives.

The choice of instrumental conditions will then be fixed since the form of the sample will determine

- the use of a polar or a non-polar stationary phase;
- the kind of column to be used, either packed or capillary, for instance;
- the detector system to be employed if the available instrumentation will allow such flexibility.

1.06 GLC OF FREE FATTY ACIDS

This section will discuss solely the problem of chromatographic separation of fatty acids. As to their identification the reader is referred to monographs (13, 14, 43, 35) where the problem is discussed in more detail.

A. Non-polar phases

The highly polar nature of fatty acids is a major

obstacle to their direct determination. James and Martin[1] observed severe tailing when attempting to separate them and later had to resort to the addition of stearic acid to the silicone oil used as the stationary phase. The action of the acid in 10% concentration was to reduce the tailing which was attributed to absorption on the supposedly inert solid support used to retain the stationary phase. Stearic acid offered only a partial solution to the problem because the chromatograms obtained still showed some difficulties. The low molecular weight acids are more polar than the higher homologs but the latter exhibit dimerization in the liquid phase which then gives rise to the opposite deviation from symmetry to that caused by absorption, namely the peaks show a leading front rather than a tail. Also, stearic acid would start bleeding from the column if separation of the higher fatty acids at higher temperatures was desired. Work by Beerthuis et. al.[36] showed that if the molar concentration of the acids is kept below 0.007 and the temperature raised to 200° C it is possible to obtain them almost entirely as monomers in the chromatographic column as demonstrated by infrared studies. This conclusion allows the separation of free fatty acids from C_{12} to C_{18} on Apiezon L at 276° C but the peaks are still far from symmetric. Another stationary phase used by Beerthuis was silicone oil plus β-anthraquinone carboxylic acid. SE-30 methyl silicone rubber may be used at higher temperatures

than Apiezon L but is non-selective and will not separate isomeric unsaturated fatty acids such as oleic and elaidic acids.

B. <u>Polar stationary phases</u>

In 1958 James used a polyester, ethylene adipate[45] and obtained good resolution of the C_{18} saturated and unsaturated acids. This marked the beginning of a new approach to the GLC of FFA with the use of polyesters as stationary phases of a more polar nature. Their coming into use more or less coincided with the increasing tendency initiated by Cropper and Heywood[5] in 1953, to prepare methyl esters of the acids and to try to separate them instead of their much more polar parent compounds. Some authors[46,47,48] however, still insisted on the direct analysis of FFA, separating low molecular weight FA using dioctyl sebacate in conjunction with sebacic acid[46] and polyesters of dibasic acids and glycols[47,48]. The new polyester phases presented the advantages of allowing better separations of unsaturated from saturated acids at lower temperatures than with other phases but had also several disadvantages such as low stability at temperatures above 200° C and transesterification with the stationary phase when the methyl esters were used as derivative compounds. The polyester columns have the advantage of greater selectivity over Apiezon L or M greases or over the silicone

rubbers on the basis of degree of unsaturation but the disadvantage of lower selectivity on the basis of double bond configuration and position[43].

The next significant development was introduced by Metcalfe[6] who demonstrated that the use of phosphoric acid-treated polyesters as the stationary phase for the separation of saturated and unsaturated FA in the range of C_4 to C_{22} was possible and that well-defined symmetrical peaks were obtained. The stationary phase so prepared also reduced the possibility of association of the carboxyl groups in the column when the free acids are injected[7]. The polyester used for the first experiments was LAX-2R-446 (diethyl glycol adipate) mixed with 2% H_3PO_4, which was shown to be heat stable, possibly because of ester formation between phosphoric acid and the hydroxyl groups of the polyester.

Some other interesting phases which have been introduced were those prepared and made commercially available by the staff of Varian Aerograph (then Wilkens Instrument & Research, Inc.)[49]. One of them, FFAP or "free fatty acid phase", is reported to be a reaction product between Carbowax 20 M (polyethylene glycol polymers) and 2-nitroterephthalic acid. A previously reported phase[37] was a product of Carbowax-terephthalic acid. 20M-TPA, another liquid phase developed at Aerograph by Jordan, G., and Byars, B., is a modification of reaction products between Carbowax and

therephthalic acid. It is reported to show good properties in separating highly polar compounds and free fatty acids.

Some authors[50] are suspicious of the polyesters as stationary phases because of the variation in their performance from batch to batch both as regards retention time and quantitative response factors for the fatty acids. This is due to the fact that the polyesters are indefinite-type of polymers whose molecular weight and terminal groups will vary, depending on small changes in reaction conditions used for their synthesis. Thus the retention times of the acids are directly proportional to the concentration of hydroxyl group ends in the polymers which require careful calibration of each column for both response factors and retention times.

To date the latest development in stationary phase technology which can still have some influence on the analysis of FFA is the introduction by Hollis[51] of porous polymer beads. They constitute a kind of borderline between the conventional support-coated column filling material and a solid which constitutes, by itself, both the liquid phase and the solid support, with a large surface area. The polymers do not behave as though they were solid adsorbents, though, and the process of chromatographic separation through them has been regarded as a "solubility" of the solutes in the porous polymer which then "assumes the partition properties of a highly extended liquid surface."

Some types give rather symmetrical peaks for the free acids but their use is limited to low molecular weight compounds because of long retention time limitations.

C. Column length

Another problem to be considered separately is the range of carbon atoms found in the acids composing the sample to be analyzed. It has been found that it is not convenient or sometimes even unfeasible to attempt complete resolution of mixtures containing both short and long chain FFA on a single column. The short chain acids are less volatile than their methyl esters and they can be separated in the free form, their low volatility preventing loss during preliminary handling operations, although they may also be determined as methyl esters. The long chain FFA do not separate well and their esters are a better choice. James[(43)] recommends several approaches to the problem among which a prior fractionation step by azeotropic distillation in toluene to obtain two overlapping groups, i.e., the C_1-C_6 and the C_5-C_{20} groups. Also possible is the use of PTGC. When detailed analysis in the range C_6-C_{22} is required he advocates the use of both polar and non-polar phases and a combination of the information gathered from the two types. The reader is referred to the original article for details.

D. Conclusions

From the preceding,one is easily led to the conclusion that the determination of FFA is fraught with difficulties imposed mainly by the characteristics of the compounds themselves, the choice of useful stationary phases and the enormous variety of composition of samples of biological origin which will bring about stringent demands on the analyst's ability to make certain that he is actually separating most if not all the components of a mixture and being able to identify and measure them quantitatively. The other conclusion is that the difficulties encountered more or less point in the direction of the preparation of derivatives which may minimize some of the requirements just discussed.

1.07 GAS CHROMATOGRAPHY OF FATTY ACID DERIVATIVES

The objective of preparing derivatives of fatty acids is to find compounds which are less polar, more volatile and perhaps more stable than the free acids. Other desirable characteristics are:

a) less or no reactivity with the solid support.

b) it should be simple to form.

c) the derivatization reaction should go to completion or near completion (minimum 93%).

d) there should be no sample loss during manipulation

or concentration of sample solution.

Esters have always been the derivatives of choice for acids in general and fatty acids are no exception. Some acids like the sulfonic acids, however, cannot be analyzed in the form of, for example, their methyl esters and the sulfonyl chlorides or sulfonyl fluorides are used instead but these are obviously an exception. For the majority of acids the esters, then, constitute the most common derivative form and the following types have been used:

1) methyl esters
2) propyl, butyl, dodecyl esters
3) phenacyl esters
4) 2-chloro-ethanol esters
5) trimethylsilyl esters

Here, again, the subdivision of FA into two 'groups' according to chain length is useful.

A. Short-chain saturated fatty acids (up to C_8 or C_{10})

Their methyl esters are much too volatile for handling and preparation without significant loss. Heavier esters, then, have been used so as to circumvent that problem. Clément and Bézard[28] converted the acids from C_4 to C_{20} to butyl esters. Langner[52] prepared the n-amyl esters of C_1-C_5 acids, while Craig et. al.[53] made a comparative study of the butyl, decyl and phenacyl esters of C_3-C_9 fatty

acids. Oette and Ahrens[54] obtained the 2-chloroethanol esters of C_3-C_9 acids but formic and acetic gave variable results.

B. <u>Long-chain saturated acids.</u>

The methyl esters have been used almost universally for acids of longer chain lengths. The differences among authors are to be found mostly in the specific procedures used for their preparation. Some workers, however, have used different derivatives. Lefort, Paquot and Pourchez[55] have studied the chromatographic behavior of methyl-, propyl-, and isopropyl esters of C_{10}-C_{18} FA and found that the methyl and isopropyl esters showed almost identical retention times on polyester (DEGS) columns. On Apiezon L the C_{10}-C_{14} esters are eluted in the order methyl-, isopropyl-, and propyl esters. The isopropyl esters, starting at C_{16} are not eluted on the Apiezon L column. Esposito[56] studied the trimethylsilyl derivatives of lauric, myristic, palmitic and stearic acids but did not present quantitative results. He also refers to an observation made by Zinkel et. al.[57] who obtained incongruous results when TMS esters of FA and resin acids were separated on polar phases. Martin[58] and coworkers made a comparison of GC analysis of methyl and TMS esters of caproic, myristic, lauric and stearic acids and found that for separation and quantitative results of n-alkanoic acids the use of TMS esters

offers no significant advantage over the use of methyl esters.

C. Gas Chromatography of Fatty Acid Derivatives

The esters of FA may also be separated on the same types of stationary phases described in Section 1.06 for the analysis of FFA. The introduction of methyl esters for GLC analysis is generally attributed to Cropper and Heywood[5] who analyzed esters up to C_{22} on silicone grease at 230° C. James and Martin[44] prepared the methyl esters from formic to octadecanoic for chromatography on Apiezon M and later James[45] employed an EGA polyester to separate C_{18} saturate and unsaturates. Since his publication in 1958 the trend has been to use a rather long array of polyesters as stationary phases. More recently, Supina[59] described the use of organosilicon polymers which consist of ethylene glycol succinate polyesters chemically modified with several types of silicones. Conditions for chromatography using several of these phases are summarized by Burchfield and Storrs[13].

The considerations made in Section 1.06 regarding the behavior and properties of the phases themselves are, of course, applicable here. As to advances in column technology, capillary columns have only comparatively recently been used for the separation of material of lipid origin. Condon[12] and Lipsky et al.[8,9,10] and also Ackman[11]

have used capillary columns successfully for the separation of FAME. In most of the work done the stationary phases are either Apiezon L or a polyester, although it has been shown that even with capillary columns the efficiency of polyester columns is unsatisfactory. Recently, however, improvements in quality of polyesters and in the coating technique for open tubular columns seem to have ameliorated this situation.

1.08 ESTERIFICATION TECHNIQUES

The previous section had the objective of demonstrating the greater use of methyl esters as derivatives of FA for GC analysis. Presently the techniques for the preparation of the methyl esters will be dealt with and an attempt shall be made to critically evaluate some of them.

A. Esterification with mineral acids

Mineral acids are used to drive the esterification reaction

$$RCO_2H + R'OH \rightleftharpoons RCO_2R' + H_2O \qquad (1.06)$$

to completion both by a drying and a catalytic action.

James and Martin[44] and James[60] are credited with being the first to use methanol-hydrochloric acid for the preparation of about 50 esters during the execution of

the works cited. James[43] describes a procedure for esterification as follows:

"The sample of fatty acid in light petroleum is evaporated to dryness in vacuo at 50° using a rotary evaporator. As soon as the last trace of solvent has disappeared, a 20-fold excess of dry methanolic HCl (0.5N) is added, and the solvent is refluxed for 2 hours on the water bath. At the end of this period the flask is removed and excess methanol removed on a rotary evaporator in vacuo. Light petroleum is then run into the flask and its contents washed out into a separatory funnel. The petroleum ether layer is washed with 0.5N sodium bicarbonate to remove mineral acid, then with water; it is dried over anhydrous sodium sulfate. When the sample is to be stored, the concentration should be kept to 1 mg per ml or less.

If the sample is originally a lipid, alcoholysis with acidic methanol can be used. James[43] also describes a suitable procedure.

"In this procedure the lipid is heated in a sealed tube for 10 hours in a 10-fold excess 2N HCl in anhydrous methanol on the water bath (the HCl-methanol solution is prepared by passing dry HCl gas into methanol that has been distilled over freshly prepared calcium oxide). At the end of the period the tube is opened, excess methanol removed rapidly in vacuo, and the residue extracted three times with 40-60° light petroleum. The solution is washed with 0.5N sodium bicarbonate and dried over anhydrous sodium sulfate. Phospholipids containing more than a few percent of plasmalogens should not be treated in this manner, since hydrolysis of the plasmalogens produces aldehydes which react further to form dimethylacetals. The dimethylacetals will give rise to peaks in the final chromatogram that will be confused with those derived from the fatty acid esters."

Stoffel, Chu and Ahrens[30] modified the above procedure in the following way:

"The esters or acids to be methylated (1 to 10 mg) are dissolved in 4 ml of 5% HCl in superdry methanol and 0.5 ml of dry benzene in a 15 ml microsublimation tube to which a condenser with a calcium chloride moisture trap is connected. The mixture is refluxed ... at 80° to 100° for 2 hours, with frequent shaking at the start to dissolve the lipid mixture. After cooling to room temperature, two volumes of water are added, and the methyl esters are extracted three times with 3 ml of petroleum ether. The pooled extracts are simultaneously neutralized and dried over sodium sulfate-sodium bicarbonate mixture for 1 hour. The esters are then quantitatively transferred with petroleum ether to a second microsublimation tube and the solvent is evaporated to dryness at reduced pressure in a 40° C water bath.

Microsublimation. After the microsublimation tube is fitted to the cold finger, a vacuum of 0.2 ± 0.15 mm of Hg is produced. The tube is then lowered into a ... bath at 60° ± 2° C for 60 minutes. The assembly is disconnected after cooling, and the sublimed methyl esters are rinsed off the cold finger with petroleum ether into a glass stoppered tube. After evaporation of solvent, the preparation is now ready for application to the gas-liquid chromatography column."

It will be noted that in the second procedure described, the final product contains not only the esters but also unsaponifiable material from the lipid. Since the sample contains both components its injection in the GC apparatus will cause the accumulation of undesirable material at the top of the column. This difficulty is eliminated in the procedure described by Stoffel at the cost of more extractions and a microsublimation procedure.

Rogozinsky used 0.5 g of the FA, added 2-10 ml of dry MeOH. The mixture is swirled, 0.5-1 ml of concentrated sulfuric acid is added and the solution boiled. The

solution is cooled and 2 ml of n-hexane added for extraction of the esters. The 20% solution obtained will be ready for direct injection. In one paper[61] he used the lower amounts of the reagents and claims that the FA above C_8 will be completely extracted. Using the higher amounts of reactants, and 5 ml of ether for extraction, 99% of the C_6-C_{18} esters will be extracted[62].

Wynne et. al.[63] employed 5% perchloric acid in methanol. Lorette and Brown[64] methylated a few acids including propionic with methanol-HCl but included dimethoxypropane (acetone dimethylacetal) in the reaction mixture. The reactions are thought to proceed as follows:

$$RCOOH + CH_3OH \xrightarrow{H^+} RCOOCH_3 + H_2O \qquad (1.07)$$

$$H_2O + (CH_3)_2C(OCH_3)_2 \longrightarrow 2\ CH_3OH + CH_3\overset{\displaystyle O}{\overset{\|}{C}}-\ CH_3 \qquad (1.08)$$

The primary function of the DMP is as a water scavenger and for each mole of H_2O formed, 2 moles of CH_3OH are introduced in the reaction mixture, so that the process is self-accelerating. There is, however, some acid-catalyzed polymerization of the acetal which gives spurious peaks on GC. Simmonds and Zlatkis[65] showed that dimethyl sulfoxide acted as an inhibitor in this polymerization reaction with a concentration of 1%. They esterified a mixture of octanoic, decanoic and succinic acids in the presence of DMSO and no polymer peaks were observed upon chromatography

under identical conditions, of the ester mixture.

Mondino[66] showed that the presence of more than 1% acid catalyst during methyl esterification of FA may result in isomerization of the acids, thereby altering the quantitative evaluation of the sample. Studies were made with 1%, 2.5%, 5% and 10% H_2SO_4 catalyst.

Vorbeck and coworkers[31] used the CH_3OH-HCl (as well as other systems) method with sublimation in a comparative study and concluded that the best method used diazomethane as shall be discussed under that topic.

Clément and Bézard[28] used sulfuric acid as the catalyst in the preparation of n-butyl esters of C_4-C_{20} acids.

Oette and Ahrens[54] used HCl-2-chloroethanol for the preparation of chloroethanol esters of short chain FA.

An interesting procedure was described by Hornstein[67]. He used an Amberlite IRA-400 anion exchanger to absorb the acids which are then methylated on the resin by the addition of anhydrous MeOH-HCl.

B. Esterification with boron trifluoride

The use of boron trifluoride as a catalyst for esterification reactions appears to have been originated with Hinton and Niewland[68] in 1932. They prepared the coordination compound of BF_3 with acetic acid, $(CH_3COOH)_2 \cdot BF_3$ and used it for esterification of ethyl, propyl, n-butyl and

n-amyl alcohols, refluxing them with the BF_3 compounds. At the time their work was published, however, they could not decide whether the catalytic effect was due to the acid-boron fluoride compound or to the reaction of this substance with some of the alcohol to form the BF_3 compound.

Toole and Sowa[69] in 1937, used the $(C_2H_5)_2 \cdot BF_3$ compound, plus acid and absolute methanol to prepare propionic, acetic esters.

Mitchell[76,80] used a BF_3-methanol reagent for esterification of carboxylic acids in an analytical method using the Karl-Fischer reagent.

Apparently the interest in BF_3 compounds for esterification purposes declined after this early work and it was not until 1961 that Metcalfe and Schmitz[20] described the use of BF_3-methanol reagent for methylation of fatty acids. The method has gained wide acceptance because of the ease of preparation, use and storage of the reagent. Other workers[70,71] have since used BF_3-methanol to transesterify materials containing acetyl groups and aromatic acids.

Schupp[50] states that the BF_3-MeOH solution of the esters cannot be injected without the extraction step because the boron fluoride will accumulate on the column and will cause decomposition of samples upon chromatography later.

Appleby and Mayne[72] developed a method for quantita-

tive determination of saturated and unsaturated FA in the range C_4^--to C_{20}^- and dibasic acids in the range $C_3^=$ - to at least $C_{12}^=$ by the use of n-propyl esters which were prepared by a technique based on that of Metcalfe and Schmitz.

It has been reported[72,73] that concentrated solutions of BF_3-methanol (50% w/v) will cause addition to double bonds. One of the same workers later reported, however, that[74] no methoxy-substituted derivative of unsaturated FA is formed when esterification is carried out for 2 minutes under reflux using a solution containing 12.5% BF_3. He still warns that if work is not carried out under carefully controlled conditions it should be recognized that methoxy substituted products may arise inadvertently.

Supina[14] has advocated the use of BCl_3-methanol in place of the BF_3 compound citing as advantages its lower vapor pressure, hence possibility of storage at room temperature with no apparent loss of the catalyst and also possibility of methylation at higher temperatures - which is desirable in the case of the higher molecular weight FA. He also claimed a higher yield, 60% in a reaction at 70° C during 15 minutes whereas the yield with BF_3 was 40%.

It must be said here that Metcalfe[9] proposed the method for the rapid preparation of FAME for GC analysis but made no effort to recover the esters quantitatively since "it was specifically designed for plant control work where large amounts of sample are available." He

states, however, that *"When all possible care is taken to make recoveries quantitative (as with costly biological samples), the recovery is well above 90%."* They report FFA remaining in the esters in amounts of approximately 1%.

In 1962, Drawert[19] used BF_3-methanol reagent for the esterification of FA produced in the stomachs of Colobinae monkeys. He distilled the contents of the stomachs in order to obtain the acids; the distillate was titrated and freeze-dried. Later, samples of the freeze-dried potassium salts of the FA were treated with sulfuric acid and BF_3-methanol reagent. After removal of the insoluble fraction, the esterified fractions were injected into a reactor placed ahead of the chromatographic column. The purpose of the reactor was to complete the esterification. Drawert's work is of special importance because it formed the main motivation for the present investigation. It will be elaborated upon in a subsequent section under the heading of 'reaction-gas chromatography'.

C. Esterification with diazomethane

Diazomethane, CH_2N_2, was discovered in 1895 by von Pechmann[77] who anticipated its applicability as a reagent in microsynthesis. It has advantages over other reagents, namely:

a) the majority of its reactions take place at room

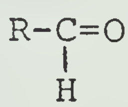
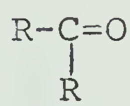
temperature.

b) a great number of its reactions are quantitative or nearly so.

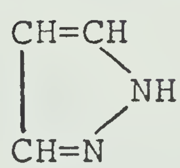
c) being a gas, excess of reagent can be easily removed from reaction mixtures.

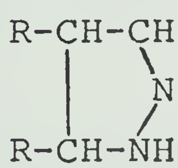
d) the reactions are simple and the products are usually purer than with other reagents.

Offsetting these desirable qualities, diazomethane is a highly toxic compound and is potentially explosive. Some of its more important reactions are summarized below. Eistert[78] has made a thorough review of the reactions of diazomethane.

1) reaction with  and  to form higher homologues and epoxides.

2) reaction with acyl chlorides to form diazoketones.

3) formation of pyrazoles,  and

pyrazolines, 

4) replacement of acidic hydrogen by the methyl group:

$$RCOOH + CH_2N_2 \longrightarrow RCOOCH_3 + N_2 \qquad (1.09)$$

The latter reaction is general and is applicable to all acids[77,79]. It proceeds at room temperature and is

usually effected in ether solutions using a small excess of diazomethane. The reaction is complete when nitrogen evolution ceases.

Roper and Ma[80] instead of preparing the reagent in a macro scale as described by von Pechmann[77] designed a microgenerator (Fig. 1.01) for its preparation.

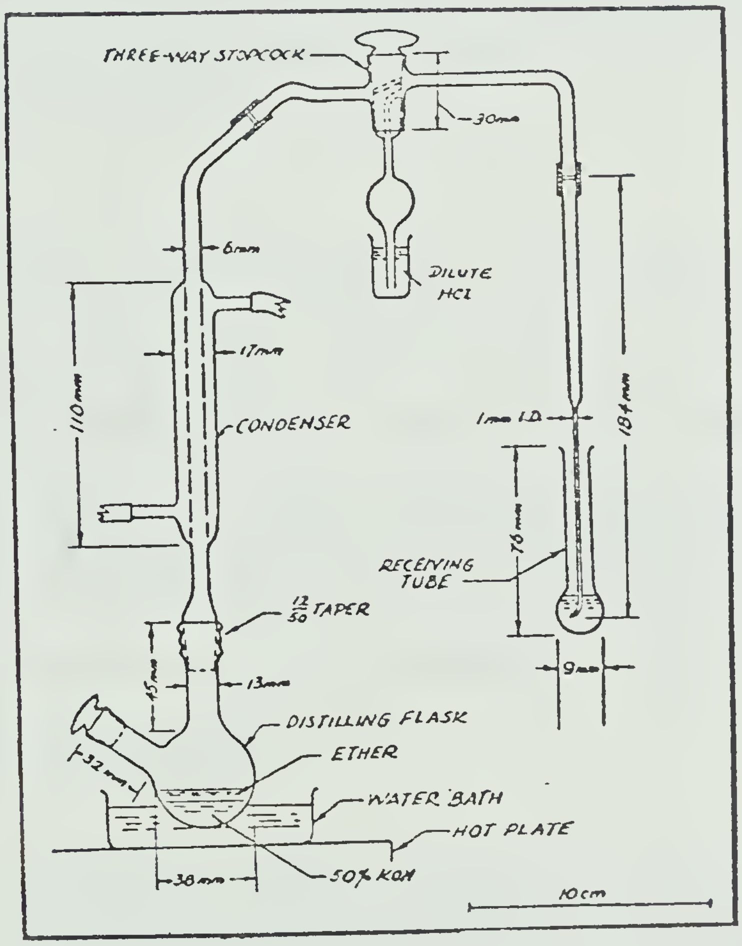


Figure 1.01 Microgenerator for diazomethane according to Roper and Ma[80].

Gaseous diazomethane was generated as follows:

"About 4 ml of 50% aqueous potassium hydroxyde was placed at the bottom of the flask and 5 ml of diethyl ether placed on top of it. The level of the water bath was adjusted to just below the level of the aqueous layer and maintained at 40-45°. N-nitroso-

methylurea, prepared according to the procedure of Adams, Vorhees, and Shriner, was added through the inlet tube, and the diazomethane was passed either directly into a suspension or a solution of the reactant, or into anhydrous ether to prepare a solution of diazomethane to be used later. The receiving vessel shown in Figure 1 was made from a 3-inch test tube whose bottom was blown out slightly. The end of the delivery tube leading into this enlarged space was bent so that the stream of gas hit the side of the tube. This arrangement was found advantageous in the case of adding diazomethane to suspensions of solids, which easily clogged the delivery tube and stopped the passage of gas if the delivery tube extended vertically into the bottom of the receiving tube."

The authors cited found out that when ether suspensions of carboxylic acids were treated with an ether solution of CH_2N_2 decanted from a lower aqueous KOH layer as in the original procedure[77] poor or no yields were obtained. Some of their results are given in Table 1.02 where this method is compared with the procedure using CH_2N_2 obtained from the microgenerator.

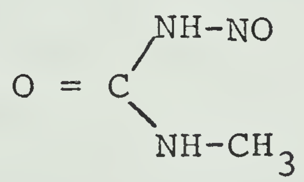
TABLE 1.02 YIELDS OF METHYL ESTERS PRODUCED BY ESTERIFICATION WITH DIAZOMETHANE IN ETHEREAL SOLUTION AND AS A GAS

	Addition of CH_2N_2 soln.			Addition of CH_2N_2 gas		
Starting material, acid	% yield ester	Starting material, mg	No. of re-crystn. or (distn.)	% yield	Starting material, mg	No. of re-crystn. or (distn.)
Anisic	26	101	0	89	72	0
Succinic	0	70	(1)	33	80	(1)
Phthalic	0	93	(1)	90	106	(1)
Oxalic	0	75	0	65	88	0
p-nitrobenzoic	18	20	1	87	86	2
p-hydroxybenzoic	22	101	2	40	87	2

(Data from Roper and Ma,[80] page 253.

The results show the advantage of passing the gas directly into the reaction mixture. Roper and Ma attribute their low yields with decanted CH_2N_2 to at least two undesirable factors: (a) the ether solution of diazomethane contained small amounts of water since it was obtained by decantation from an aqueous KOH solution; (b) the requisite quantities of CH_2N_2 solution required vessels of relatively large volume which tends to reduce the recovery of the product. They found diazomethane to be a convenient and safe reagent to be used on a microscale. The only precaution they observed was the carrying out of all work in a well ventilated hood.

It is to be noted that Roper and Ma used N-nitrosomethylurea, as a precursor of diazomethane. von Pech-



mann[77] used nitrosomethylurethane, nitrosomethylurea having been described by Arndt[81]. N-nitroso-β-methylamino-isobutylmethyl ketone[82]; 1-methyl-1-nitroso-3-nitroguanidine[83] and N-nitroso-methylacetamide[84] have been used, but they are not considered ideal materials.

De Boer and Backer[85] proposed the use of p-tolylsulphonylmethyl nitrosamide, CH_3-C_6H_5-$SO_2N(NO)CH_3$. In the same paper (ref. 11) they promise a discussion of the decomposition of diazomethane but a search in Chemical Abstracts

from 1949 to date did not reveal its publication. More recently, Moore and Reed[86] proposed the use of bis-(N-methyl-N-nitroso) terephthalamide (70% in mineral oil, available from the Explosives Dept., E. I. duPont de Nemours, U.S.A., under the trade name EXR-101) as a precursor:

$$\begin{array}{c}NO\\ \diagdown\\ N\\ \diagup\\ CH_3\end{array}\!\!-\overset{\displaystyle O}{\overset{\|}{C}}-C_6H_5-\overset{\displaystyle O}{\overset{\|}{C}}-\!\!\begin{array}{c}NO\\ \diagup\\ N\\ \diagdown\\ CH_3\end{array} + 2\ NaOH \longrightarrow 2\ CH_2N_2 +$$

$$CO_2Na-C_6H_5-CO_2Na + 2\ H_2O \qquad (1.10)$$

In the field of FA analysis by GC, diazomethane has been used by several workers. James and Martin[44], as early as 1956 used diazomethane for the preparation of methyl esters of several FA at 0° C. Schlenk and Gellerman[29] applied the method of de Boer and Backer[85] in the preparation of CH_2N_2 and $C^{14}H_2N_2$. Their esterification technique was described as follows:

> *"<u>Apparatus</u>. The apparatus consists of three test tubes with side arms. A stream of N_2 is saturated with ether in the first tube (16x150 mm) and carries CH_2N_2 generated in the second tube into the third tube (both 15x85 mm) where the esterification takes place. The side arms (7 mm in O.D.) are bent downwards and reach close to the bottom of the next tube. They are drawn out at the ends to approximately 1 mm in outer diameter. Rubber stoppers are used for connections.*

Esterification. *The flow of N_2 through the ether tube 1 is adjusted to approximately 6 ml per minute. Tube 2 contains 0.7 ml of Carbitol, 0.7 ml of ether and 1 ml of a solution of 6 g of KOH in 10 ml of water. Between 5 and 30 mg of fatty acids dissolved in 2 to 3 ml of ether which contains 10% methanol is placed in tube 3. About 2 moles of MNSA or M-C^{14}-NSA per milliequivalent of fatty acids is dissolved in 1 ml of ether and added to tube 2. As soon as a yellow tinge becomes visible in tube 3 against a white background, this tube is disconnected and the slight excess of CH_2N_2 in it is consumed by adding acetic acid diluted with ether, or removed by a stream of N_2. The whole procedure requires 10 to 12 minutes."*

Schlenk and Gellerman[29] made interesting observations concerning the effect of the presence of solvents and their chemical nature, on the rate of esterification. Alcohol-free solutions of CH_2N_2 in ether were used but they report repeated failure to achieve complete esterification of FA within 30 minutes. Water (0.133 M) and ethanol (0.267 M) showed effects equal to that of methanol whereas ethyl acetate, chloroform and heptane were not as effective. The addition of methanol increased the rate so that at a concentration of about 2 M it became too rapid to be measured. This is in remarkable contrast to the same reactions run in pure ether, at 20° C, where the esterification is still incomplete after 1 hour even with excess of diazomethane known to be present. Table 1.03 shows results as reported in their work.

In Fig. 1.02 the curves depict the rates of esterification as a function of concentration of solvents used.

The action of methanol was considered by Schlenk and

TABLE 1.03 ESTERIFICATION OF PALMITIC ACID WITH $C^{14}H_2N_2$

Solvent	Time in min.	% radioactivity (total) found in: Starting point	Front line	% Ester yield
10% CH_3OH in ether (v./v.)	10	none	none	100
As above	30	0.15	none	100
As above[a]	30	0.4	none	100
Ether	30	0.5	1.0	80
CH_3OH[b]	30	none	none	20

a - esterification tube scratched with sand.

b - yellow color did not appear in esterification tube.

(From Schlenk and Gellerman[29].

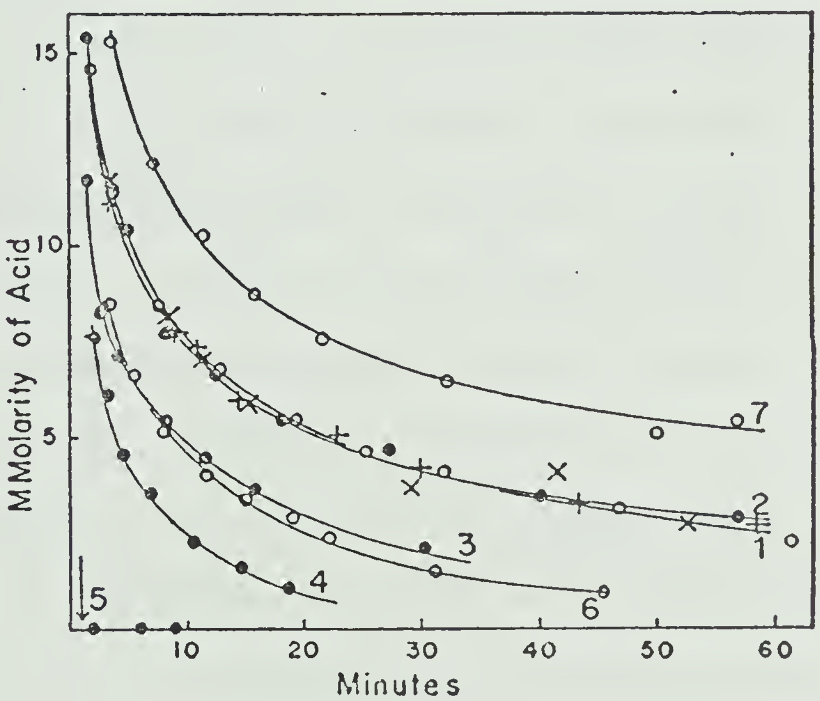


Figure 1.02 Esterification of fatty acids with diazomethane.

Temperature, 21° C; initial molarity of acids, 0.027; of CH_2N_2, 0.06; solvent ether, with cosolvents as specified.

1. *Stearic, myristic, and linoleic acids*
2. *Stearic acid, 0.0267M CH_3OH*
3. *Stearic acid, 0.133M CH_3OH*
4. *Stearic acid, 0.267M CH_3OH*
5. *Stearic acid, 2.67M CH_3OH*
6. *Stearic acid, 0.133M H_2O*
7. *Linoleic acid at 1° C.*

Gellerman to be essentially a catalytic one although promoting or adverse effects were apparent and dependent on its concentration, which the authors recommend to be under control. The experiments shown in Table 1.03 demonstrate the assumption that methanol added does not participate in the reaction and also show the non-existance of sideproducts of the esterification reaction. Another important observation made was the formation of polymers as evidenced by the appearance of turbidity and finally white flakes in nearly all reaction mixtures after 20-30 minutes.

Stoffell et al.[30] previously reported that unsaturates often undergo side reactions with diazomethane, and catalysis of inter-esterification, addition products formation with activated double bonds and triple bonds have also been reported. Schlenk and Gellerman did not find evidence for these claims when using special samples of lipids. They also did not observe pyrazoline or cyclopropane derivative formation. No structural changes were observed when linolenic or arachidonic acids were esterified.

A quantitative comparison of methylation techniques was made by Vorbeck et al.[31]. They studied four methods for the esterification of fatty acids. The methods employed diazomethane; CH_3OH-HCl with micro sublimation; CH_3OH-HCl on ion-exchange resin; and BF_3-CH_3OH. Their results indicate that the choice of a methylation procedure depends on the nature and composition of the sample. The

greatest variation among the methods was observed in the mixture of lower molecular weight acids which are best esterified using diazomethane. They do not report any significant loss of polyunsaturated FA as a result of treatment with diazomethane, and conclude their work by esterifying a mixture of both low and high molecular weight acids (C_4 - C_{20}) with CH_2N_2 using the procedure of Roper and Ma. The standard deviation found for this method was 0.72%. Tables 1.04, 1.05, 1.06 show their reported results. It is evident from the data that the diazomethane method shows the lowest standard deviation of all methods studied when applied to the methylation of higher molecular weight fatty acids. Although the paper by Vorbeck and co-workers is so far the only comparative study of the most common methods of esterification available, it is not the most complete. In our view the work of Schlenk and Gellerman though more limited in scope, brought to light some interesting aspects of the diazomethane esterification, one of which is the 'solvent effect'. It is not clear why Vorbeck et. al. did not take that into account in their study which was published at a much later date. For practical use, then, one is inclined to pay more consideration to the recommendations of Schlenk and Gellerman.

TABLE 1.04 RECOVERY OF METHYL ESTERS OF LOW MOLECULAR WEIGHT F.A.

Acid	Weight per cent			
	Added	$CH_2N_2^a$	CH_3OH-HCl on resin[a]	CH_3OH-BF_3^a
Butyric	38.7	38.6	4.0	20.1
Valeric	30.0	29.8	14.8	24.4
Caproic	31.2	31.6	29.6	30.4
Std. dev.		±0.32%	±26.5%	±13.8%

a-Average of four determination on each sample.

TABLE 1.05 RECOVERY OF METHYL ESTERS OF HIGH MOL. WT. FATTY ACIDS

Acid	Weight per cent					
	Added	$CH_2N_2^a$	CH_3OH-HCl on resin[a]	CH_3OH-BF_3^a	MeOH-HCl+micro-sublimation[a]	
					Residual[b]	Found
Caproic	7.3	7.3	6.2	6.7	c	c
Caprylic	9.0	9.1	7.9	8.4	c	c
Myristic	14.2	14.7	13.8	13.9	17.0	17.8
Palmitic	16.1	15.9	15.7	15.6	19.2	19.6
Stearic	17.8	17.6	18.7	18.0	21.3	21.0
Oleic	17.8	17.6	17.7	17.4	21.3	21.0
Linoleic	17.8	17.8	17.8	17.8	21.3	20.7
Std. Dev.		±0.25%	±0.77%	±0.46%		±0.52%

a-Average of four determinations on each sample

b-Residual composition calculated on basis of: myr., palm, stearic, oleic and linoleic acids.

c-Caproic and caprylic acids lost during sublimation.

TABLE 1.06 RECOVERY OF METHYL ESTERS[a] OF FATTY ACIDS USING TWO COLUMNS

Acid	Added	Recovered[b]
Butyric	14.8	15.5
Valeric	11.4	12.0
Caproic	9.8	9.8
Caprylic	9.6	8.6
Myristic	12.4	11.6
Palmitic	11.9	11.3
Stearic	9.5	10.2
Oleic	10.5	10.1
Linoleic	10.0	10.8
Std. dev.		±0.72%

a-Prepared by reaction with diazomethane

b-Average of four determinations

(All data from Vorbeck et.al.[78].

1.09 REACTION-GAS CHROMATOGRAPHY AS AN ALTERNATIVE TECHNIQUE FOR ESTERIFICATION

A. Brief Review

In 1955, Kokes, Tobin and Emmett[15] introduced a new dimension in the study of chemical reactions when they attached a micro-reactor to a gas chromatograph. In their own words,

> *"Recently, it occurred to us that by attaching a reactor unit to the top of a chromatographic column, one could inject small quantities of reactant into a suitable carrier gas, pass the mixture over a catalyst and analyze the exit gas from the reactor*

by passing it directly into a chromatographic column. We have now successfully completed a number of catalytic runs by this technique. The present note is a brief description of the apparatus used, the results obtained, and the potential application of this procedure."

... "The possible applications of this micro-technique are so numerous and obvious as to need little explanation. The catalyst chamber may be replaced by an empty tube for thermal reactions or for reactions in the presence of radiation. The column may be varied according to known techniques to permit the analysis of a variety of products ..."

Emmett's apparatus is shown in Fig. 1.03. It was used in their first paper for the study of the decomposition of 2,3-dimethylbutane by a cracking catalyst and for radioactive tracer experiments using a 50-50 mixture of C_3H_6 and $C_2^{14}H_4$. Later, Hall and Emmett[16] improved the instrument with the addition of an elaborate sample introduction system. The concept was further developed by Emmett[87,88] and other workers quickly realized the potential of the technique, there following the appearance of several papers in the literature.

In 1960, Friedrich Drawert and coworkers[17,18] introduced the terminology 'Reaction-Gas Chromatography'. It is thus accepted that Drawert coined the now adopted designation for the technique, although the original concept was applied and developed by Emmett and coworkers. Presently the technique has expanded into a rather broad "ancillary" technique of gas chromatography[21,22,23,24]. Beroza and Coad[26] in 1966 stated that there were almost 500 publica-

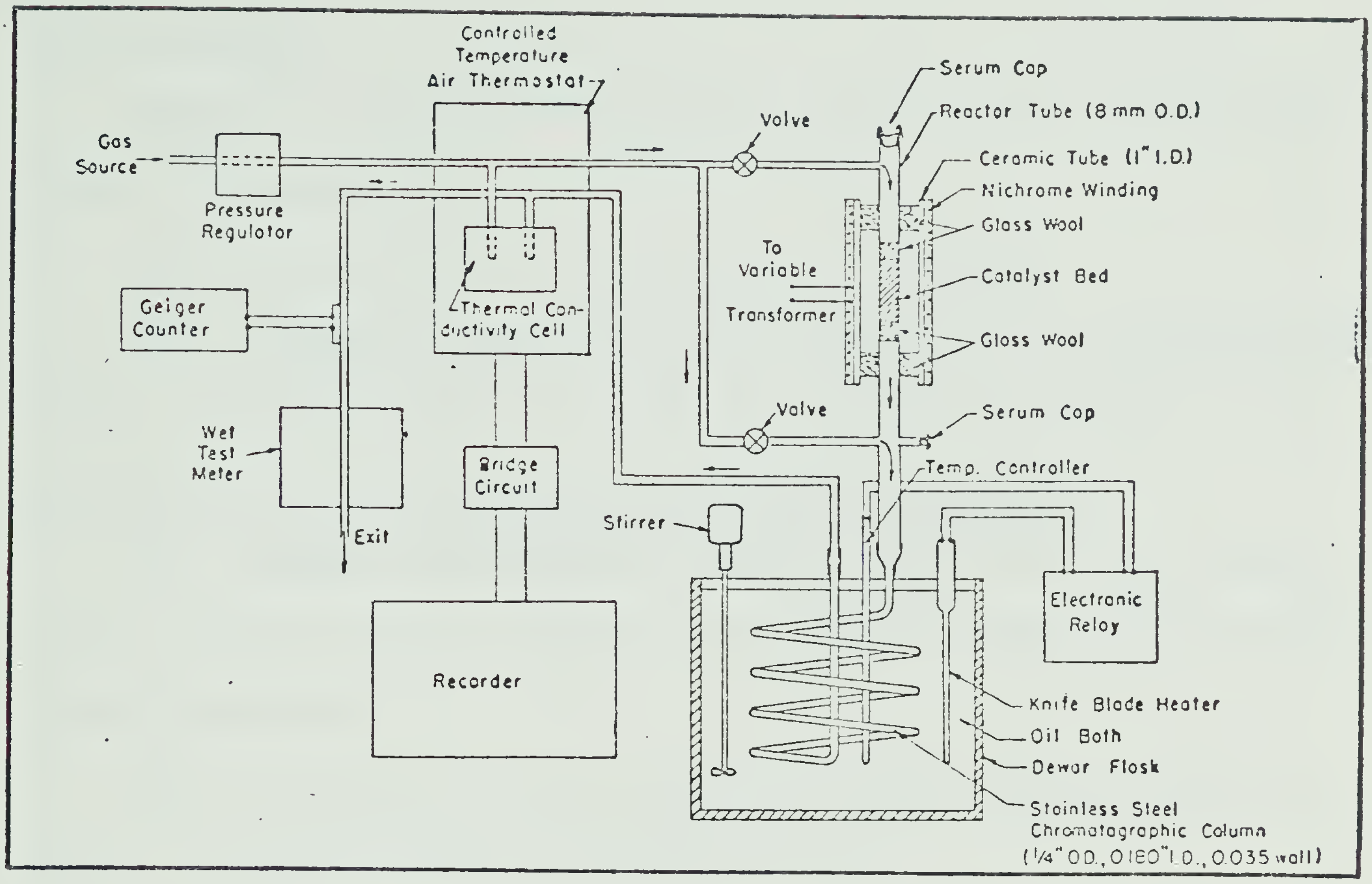


Figure 1.03 Microreactor and chromatographic product analyzer unit of Kokes, Tobin and Emmett[15].

tions on methodology that may be classified as reaction-GC: hydrogen reactions; pyrolysis GC; elemental analysis; class reactions; subtractive processes; combination of two or more reactions, etc. They also published a thorough survey of the field with detailed bibliography[25].

The accepted definition now[25] is that if a reaction is completed before its products are injected on a GC instrument, the process is NOT to be considered reaction-GC. However, if the reaction products are held within a closed system for sudden release, the procedure does fall within

this category.

Ettre[89] cites the following reasons for the utilization of reaction-GC:

(a) A reaction or a catalyst is studied.

(b) A sample which otherwise could not be investigated by gas chromatography is modified in order to permit a GC analysis or a GC characterization of the sample.

(c) Sample components, either before or after separation, are modified in order to facilitate their qualitative identification.

(d) Fractions in the column effluent are modified in order to enhance detector sensitivity.

Beroza and Coad[26] state that

> *"the combination of rapid reaction (usually at elevated temperatures) and gas chromatographic analysis offer the chemist an opportunity to try and develop vapor-phase technology useful for analytical purposes. Thanks to the excellent detection and measuring systems used in gas chromatography, many reaction gas chromatographic methods yield quantitative data, and because the sample is processed in a closed system, transfer or handling losses or difficulties are not usually experienced. As in conventional GC minute amounts of substance are readily analyzed. This feature is of special interest these days because gas and other forms of chromatography have greatly facilitated the isolation of pure substances in very small amount. Accordingly, the need to engage in time-consuming preparative gas chromatography for identification or structure determination of these substances may be held to a minimum if suitable reaction gas chromatographic procedures are devised. For rare compounds or potent physiologically active substances that are available only in minute amount, such analyses would be invaluable."*

Ettre[89] and Steingaszner[27] discuss the possible variations of reaction-GC from an operational point of view: the principal possibilities are shown in Fig. 1.04. In the present work, we shall be concerned with the situation depicted in Fig. 1.04-a which consists of the methods where a reaction is carried out to study either the change in the reaction products due to modification of the reaction parameters or the behavior of a catalyst used to bring about a specific reaction. These methods are usually termed microreaction techniques, because as compared to pilot-plant operations the reaction is carried out in a micro scale. Steingaszner[27] classifies the same type as "intermittent or periodic microreactor operations (slug or pulse technique)" and includes there the classical work of Emmett.

B. A more detailed examination of Drawert's work

The first application of reactor techniques made by Drawert was in connection with his studies of alcohol content of wines[17]. He used a 'reaction vessel' (Fig. 1.05) in place of the injection port of a gas chromatograph. The reaction chamber was filled with $NaNO_2$/Sterchamol (1:1) mixture. Wine was injected and water from the reaction was prevented from going into the column by reaction with CaH_2 in a (1:1) mixture with Sterchamol placed in another chamber just before the entrance to the column. The alco-

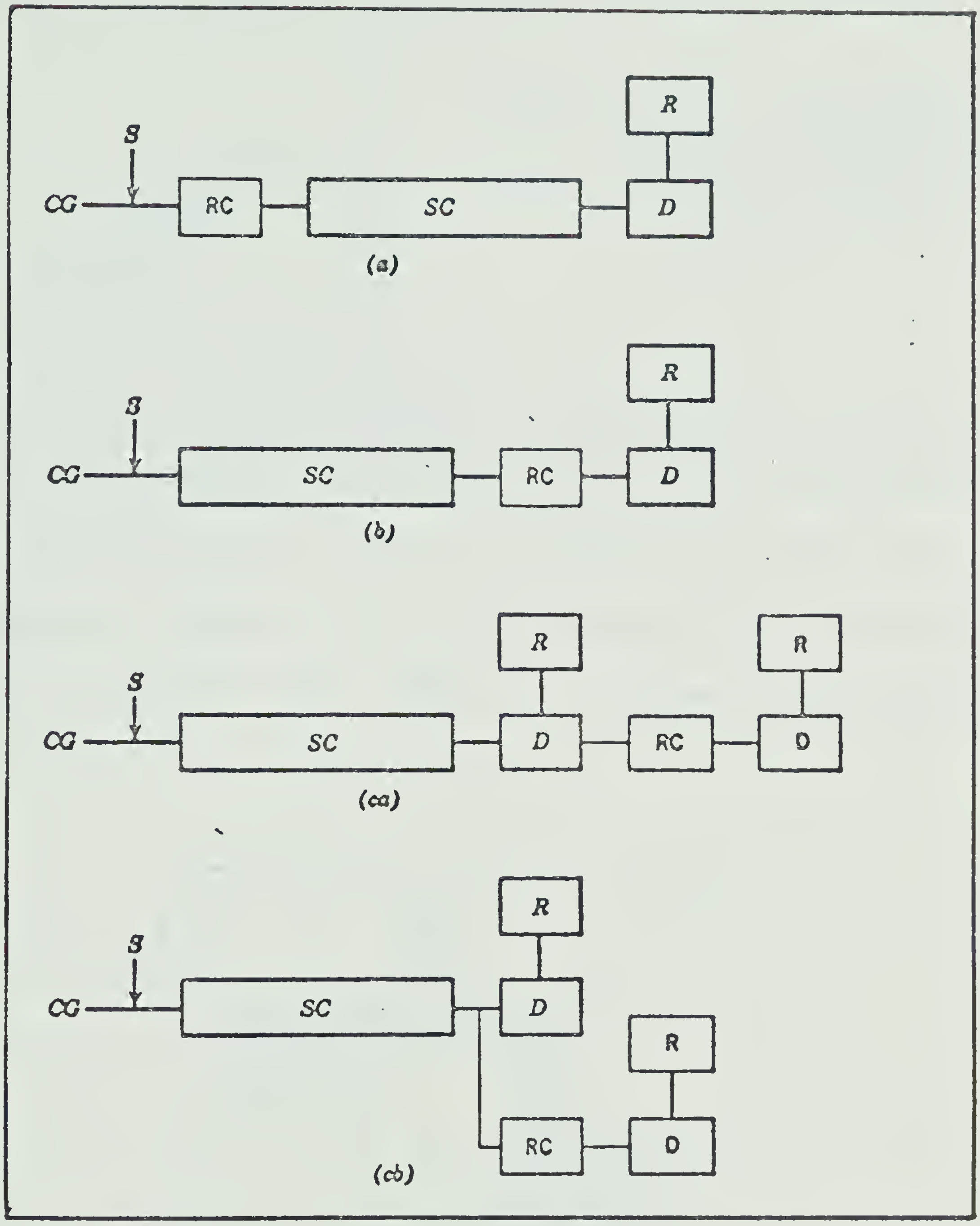


Fig. 1.04 (From Ettre[89]).

Block diagrams of the various principal possibilities for reaction gas chromatography. The basic parts of the standard gas chromatographic system are indicated with heavy lines and symbols in italics. CG, carrier gas; S, sample; SC, separation column; D, detector; R, recorder (readout); RC, reaction chamber (reactor); D and R, detector and recorder after the reactor.

hols were determined in the form of their nitrites.

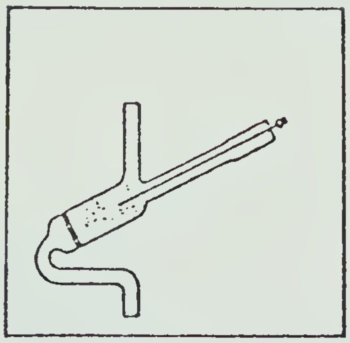


Figure 1.05 Reaction essel used by Drawert[17]. Used in place of injection port.

In 1962 Drawert[18] made an interesting application of reaction-GC to the esterification of fatty acids as has been seen in Section 1.02-B in connection with the use of BF_3-methanol reagent. Fig. 1.06 depicts his system as described in Ref. (22). Fig. 1.07 shows in more detail the

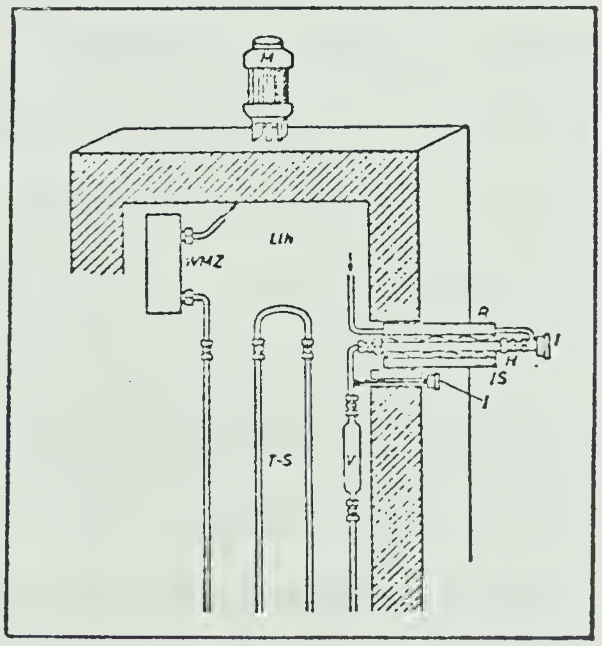


Figure 1.06 Gas chromatograph with reactor, Drawert[29].

Lth, air thermostat; M, motor with fan; TS, separating column; WMZ, TC cell (Siemens); I, injection points; R, reactor, with H, heating and IS, insulation; V is the CaH_2 fore-cell.

construction of the reactor.

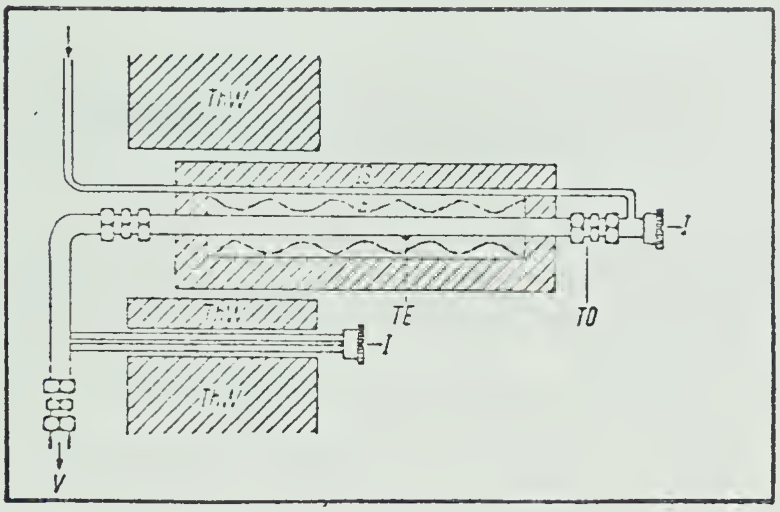


Figure 1.07 More detailed view of the reactor in Figure 1.06. TE, thermoelement; TD, Teflon fitting; H, heating.

The reactor tubing itself was made of ceramic, 24 cm long and was filled with quartz wool. Heating was accomplished by an independent heater and the whole system was placed in an opening on the GC instrument's oven wall. A separate injection port could bypass the reactor for introduction of samples just prior to a special chamber which contained CaH_2 in a 1:1 mixture with Sterchamol. Drawert had previously demonstrated(17,18) that calcium hydride did not affect esters but did retain water and acidic residual. The purpose of the CaH_2 chamber was then to retain undesirable substances prior to their entrance into the chromatographic column. It was possible, then, to inject samples of the products of an esterification reaction without prior extraction of the esters, thereby avoiding possible losses of the more volatile components of the mixture. In this case the reactor's role was to

bring the esterification reaction to completion and to provide reproducible conditions for analysis.

1.10 THE PRESENT PROBLEM

A. Preliminary considerations

From the previous survey of the literature with regard to (a) the methods applicable; (b) the esterification techniques for fatty-acids; (c) the techniques of reaction-gas chromatography, several points become apparent:

1) Gas chromatography is undoubtedly a superior method for the analysis of fatty acids in the form of suitable derivatives, being capable of application even to free fatty acids in some cases.

2) In most cases the esterification procedures had to be followed up by an extraction procedure with potential loss of the esters involved, especially those of low molecular weight.

3) All the esterification procedures examined were applied to samples prior to their injection in the GC instrument.

4) Even in Drawert's elegant application the purpose of the reactor was to complete an esterification reaction which had actually been initiated outside the GC instrument.

B. The problem

An investigation of the possibilities of application of reaction-GC techniques without a partial esterification of the free fatty acids outside a reactor system was the basis for the research to be covered in this thesis. Fatty acids both of lower and higher molecular weight will be esterified to their methyl esters by means of a suitable reagent and/or catalyst system, all being injected into an all-glass reactor.

The first reagent chosen was BF_3-CH_3OH prepared according to Drawert and Metcalfe.

The main variables to be investigated would be the time of residence of the esterification mixture plus reagents inside the reactor, governed by the flow rate of the carrier gas; and the reactor temperature plus its packing (if one is used). Those would possibly provide data for the optimization of the reaction-GC system so as to obtain reproducible quantitative results of analytical value.

The system past the reactor will be a conventional GC instrument using any convenient type of detector, with means for the ready adaptation of the reactor to the entrance of the GC column.

The main requirements of the reactor were deemed to be good temperature control, provisions for flow control, accessibility and ease in assembly and dismantling opera-

tions during change of catalysts, packing, etc. The system is shown in its generalized arrangement in Fig. 1.08.

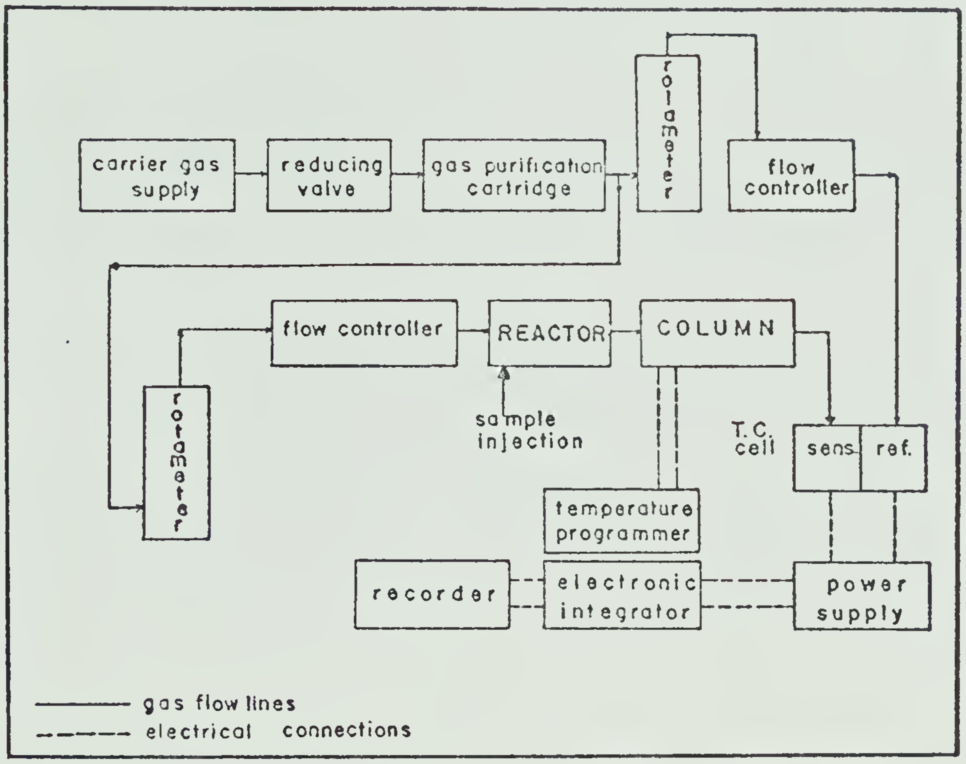


Figure 1.08 Diagram of a tentative arrangement of components for a reaction-gas chromatographic system.

It was anticipated that esterification will be a gas phase reaction and good mixing of the reagents as well as good control of the temperature of the reaction vessel will be essential. An attempt will be made to evaluate reactor design and operation necessary to accomplish a gas phase esterification reaction.

The chromatographic separation of the reaction products after the esterification proper will have to be studied from the point of view of the nature of the products themselves which will dictate the choice of liquid phase needed for optimum resolution and peak symmetry. The latter in turn

are essential for the use of electronic integration for the quantitative evaluation of the chromatogram.

Programmed temperature techniques were deemed essential following a recommendation by Herb, Magidman and Riemenschneider[84] concerning the quantitative analysis of fatty acid methyl esters. Due attention will be also paid, wherever possible, to the recommendations of Horning, Ahrens and others[90] concerning the quantitative analysis of fatty acids by GC, in a special report of the "Fatty Acid GLC Committee" of the National Heart Institute program (U.S.A.).

2. ESTERIFICATION OF FATTY ACIDS BY REACTION-GC WITH BORON TRIFLUORIDE-METHANOL AS A REAGENT

2.01 INTRODUCTION

As was stated in Section 1.10.B of chapter 1, the present investigation was derived from the work of Drawert[22] et alii who first used a reactor to complete the esterification of fatty acids initiated outside the chromatographic system. It was decided to find out whether the external esterification step could be totally dispensed with, the esterification mixture being injected without prior manipulation into the reaction chamber which would then have the task of bringing the esterification to completion.

It will be seen that the efforts in that direction using BF_3-methanol were to prove unsuccessful and after a series of experiments this reagent was abandoned in favor of a more promising one. The following is an attempt at summarizing the experimental work done and the possible causes of failure.

2.02 EXPERIMENTAL

Apparatus

In the work with boron trifluoride, the reactors used were designed and modified along with the development of the ideas applied. Appendix III should be consulted for a

description of the two configurations used for the reactor-column oven- detector, namely, configurations "A" and "B".

Preparation of the reagent

The reagent was prepared according to the procedure of Metcalfe and Schmitz[20]. Boron trifluoride was Matheson C.P. grade, contained in a lecture bottle. The gas was slowly bubbled through dry methanol in an Erlenmeyer flask placed on the pan of a triple beam balance. The weight of the flask and its contents was observed as a guide in indicating when the desired approximate wt./wt/ percentage was reached. The first experiments were performed using Teflon tubing connections between the lecture bottle, a glass trap and the Erlenmeyer but later an all glass system was used to avoid contact of the gas with Teflon and thus remove a possible source of contamination. The final concentration of the reagent was approximately 140 g BF_3/liter methanol.

Packing of reactor

A Pyrex glass wool plug was introduced in one end of the reactor to retain the CaH_2 particles which were loosely packed into the tube to form a 4-5 cm zone. The particles were of 30/60 mesh in size and were ground in an alumina mortar and sieved under a fume hood. No provision was made at this stage to prevent contact of the calcium hydride with atmospheric air. The experiments were performed in

winter and it was wrongly assumed that the relative humidity was low enough as to make acceptable the grinding, sieving and packing of CaH_2 exposed to air for the preliminary runs. Later all these operations were performed under a dry nitrogen atmosphere in a dry box.

<u>Experimental conditions</u>

The chromatographic column used was a 2-meter, ¼" O.D. stainless steel tubing packed with neopentyl glycol succinate polyester 10% wt./wt. on 60/80 Chromosorb W AW. The column was operated at room temperature (25° C). The reactor was maintained at 175° C (according to Drawert). The TC cell block was thermostated at 200° C and the carrier gas, helium, flow rate was 20 ml/min. Other column packings such as Porapak Q and Q-S were tried but the retention times for high molecular weight esters which were to be studied in later stages of the experimental work proved to be too long for practical use. In the experiments a Hamilton #701 microliter syringe was used. The reagent was drawn into the syringe followed by an appropriate amount of the acid to be esterified. Both were then injected through the septum on the reactor.

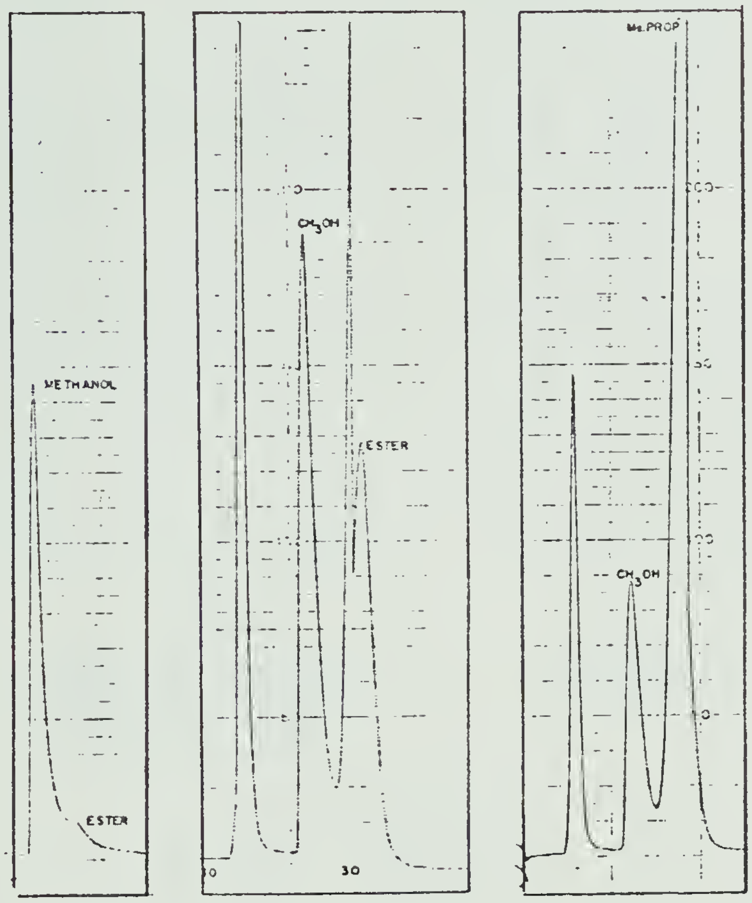
2.03 RESULTS AND DISCUSSION

Figure 2.01-a shows a typical chromatogram obtainted when CaH_2 was being prepared in the fume hood (Section 2.02,

packing of reactor). Some acid was esterified as evidenced by the small ester shoulder on the methanol peak. Retention times for comparison purposes were previously obtained by individual injections of the pure compounds. Injection of methanol and propionic acid without the catalyst did not produce an acid peak, apparently showing total absorption of the acid by the CaH_2 zone. In another experiment an 'external esterification' was performed using 0.2 ml of reagent plus 0.5 μl of acid mixed in a small beaker and 0.2 μl of the resultant solution injected after a rest period of about 2 minutes. No ester peak appears on the chromatogram but vaporization loss of the volatile ester might have occurred since no precaution was taken to prevent it.

The experiments carried out using CaH_2 prepared and packed under dry box conditions were more successful. Fig. 2.01-b shows the first chromatogram obtained with the new technique. It is seen that an attenuation of 10 was used for the methanol peak but 30 had to be used for the ester peak. Resolution is poor but the relative areas of the two peaks, as contrasted to the previous experiments indicate clearly that methanol was consumed in the reaction. In the subsequent experiments confirmatory runs were made using propionic acid, Figure 2.01-c; then butyric acid, Fig. 2.01-d and a mixture of propionic, butyric and hexanoic acids, Figures 2.01-e and 2.01-f. In all runs, 1 μl of acid plus 5 μl of catalyst were injected. In Fig. 2.01-f the

methanol peak now appears as a small shoulder on the leading edge of the methyl propionate peak. Judging from the size of the peak, it was almost depleted in this experiment.



(a) (b) and (c)

Figure 2.01

(a) typical chromatogram showing ester shoulder on methanol peak. (b) and (c) chromatograms showing relative proportions of methanol and methyl propionate peaks, as contrasted to (a)

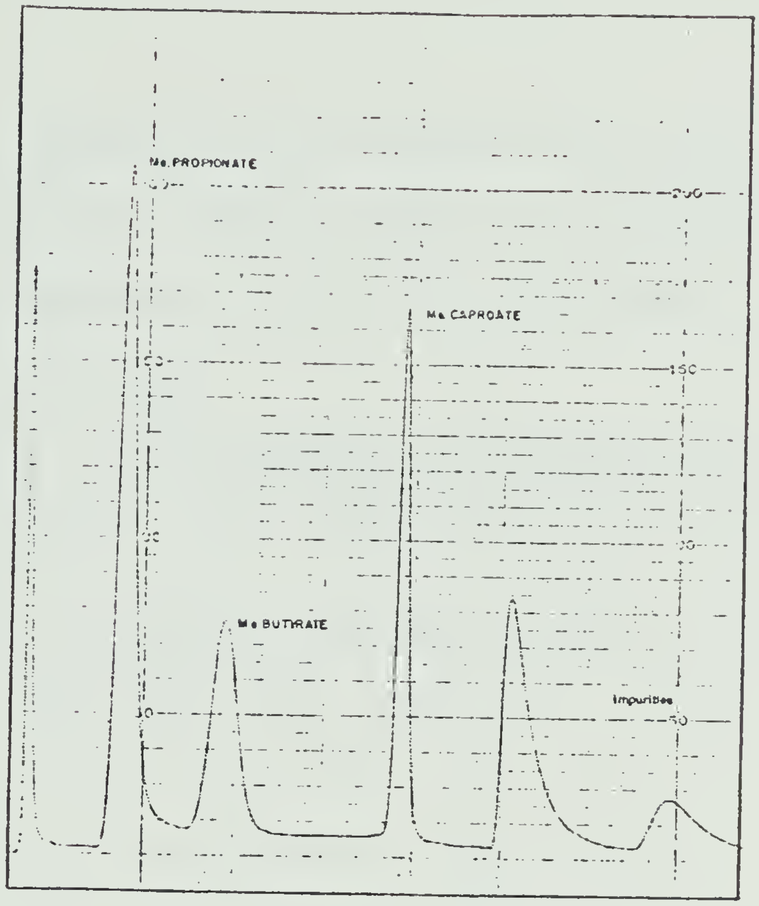
From a qualitative point of view, the previous experiments demonstrate that it is possible to accomplish esterifications entirely by a reaction-gas chromatographic procedure, without a previous partial external esterification step. It was necessary now to put these experiments on a quantitative basis to find out:

a. The extent of the esterification, or the percentage yield.

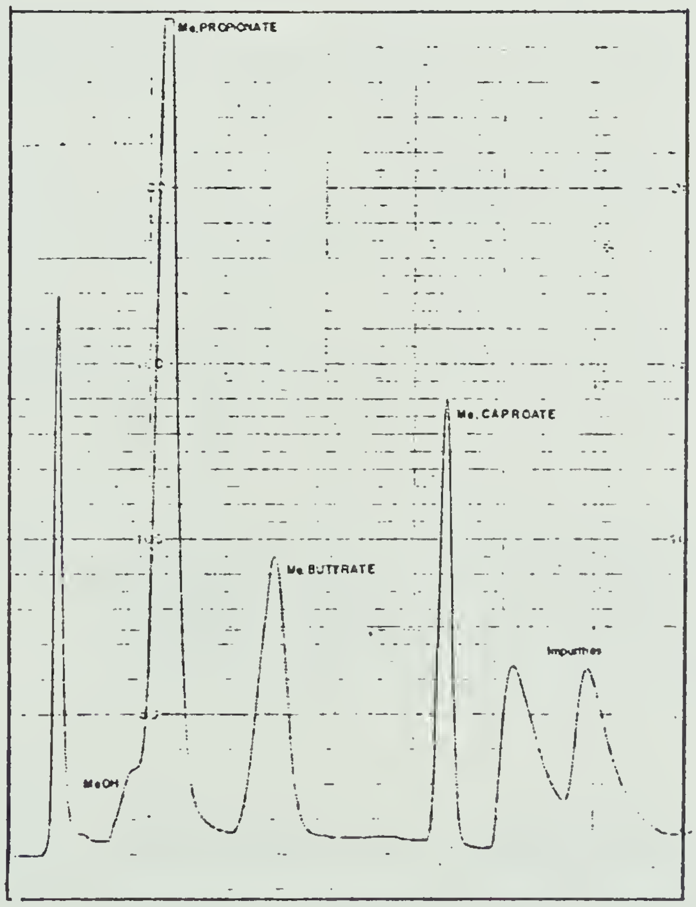
b. The effects of reagent concentration; temperature of reactor; and residence time in the reactor.

Figure 2.01 (continued)

(d)
same as (b) and (c) using butyric acid



(e)
mixture of C_3,C_4 and practical grade C_6 acids



(f)
small methanol shoulder on methyl propionate peak showing nearly complete consumption of the alcohol in the reaction

c. The occurrence of possible side effects such as transesterification or decomposition of stationary phase by the BF_3-methanol not totally retained in the calcium hydride zone.

At this stage, however, an unexpected phenomenon started to appear on the chromatograms. Fig. 2.03 shows the abnormality, which was a peak with an unusual shape, having a sharp leading edge and a long, drawn-out tail which has a long elution time and therefore interferes with the elution of other peaks, as well as their integration. The abnormal

Figure 2.02 Arrow shows the extraneous abnormal "peak" appearing on the chromatograms in place of the sharp ester peak.

peak was at first attributed to exhaustion of the CaH_2 zone in the reactor. Several efforts were made to determine the nature or cause of that peak. Other possible causes would

be the presence of water resulting from the esterification and not retained by the reaction zone or organic decomposition products. An injection of a water-acetone mixture, however, failed to produce the extraneous peak, or 'plateau peak', as it was designated. The catalyst might also be causing the problem because when pure BF_3-methanol was injected, the peak reappeared in the form shown in Fig. 2.03.

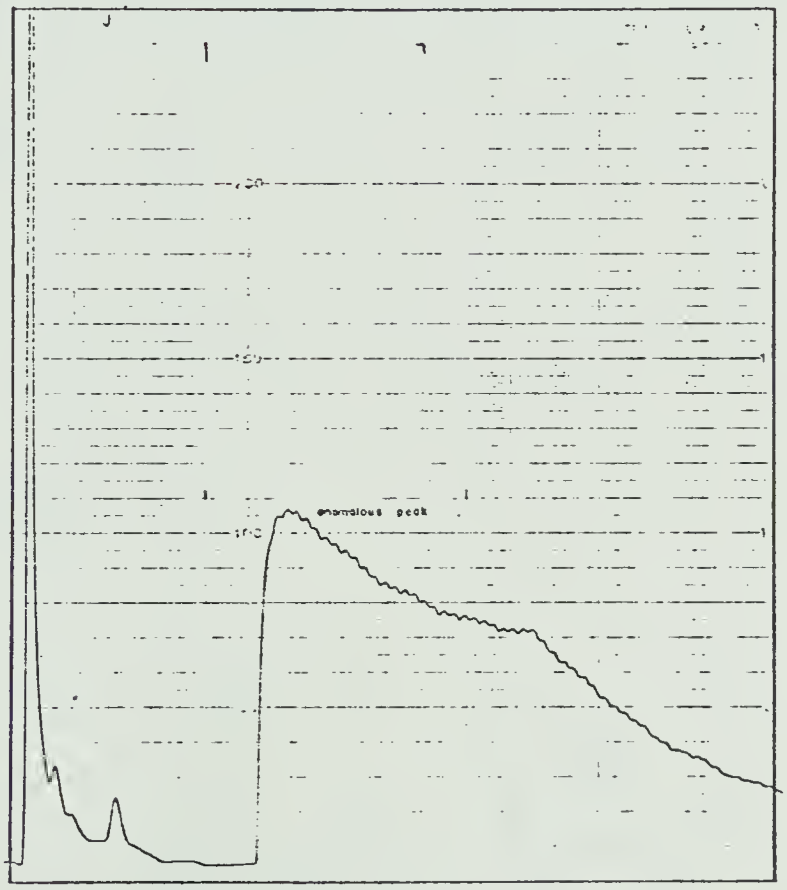


Figure 2.03 Shape taken by the abnormal peak upon injection of boron trifluoride-methanol reagent.

It was then decided to try the trapping and mass spectrometric analysis of the material which was giving rise to the 'plateau' peak. Runs were made identical to previous ones and the effluent was trapped in 1.5 mm I.D. Pyrex glass

traps immersed in a dry ice-acetone bath[100,102]. Following the trapping operation, the traps were placed in a beaker with dry ice fragments and sealed with a blow torch, the operations being executed as rapidly as possible. The mass spectrometric runs were made in the Department of Chemistry in the MS laboratory[101], using an AEI MS 9 high resolution machine although a lower resolution instrument should have been used. Figure 2.04 depicts the bar graphs of the spectra

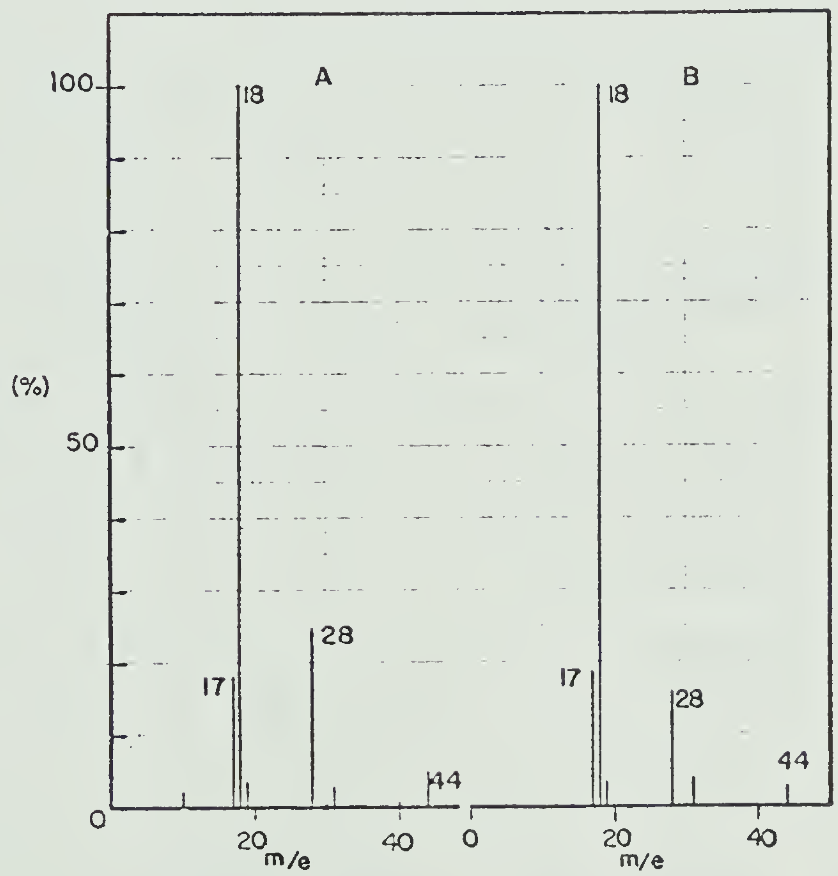


Figure 2.04 Mass spectra bar graphs showing predominance of water peaks.

of the peaks of interest. They indicate the presence of water, m/e 17 and m/e 17 is probably due to OH^+. m/e 44 is due to carbon dioxide. In both spectra the base peak is water. The scans were examined for the presence of ions corresponding to BF_3 or some decomposition products thereof

containing boron or fluorine and also for organic impurities but none were found. Thus, it is not certain that the results of the mass spectra are conclusive. The possibility exists that the original component (or components) of the extraneous peak may not have been adequately retained by the traps employed. No means was used to prevent aerosol formation and if it occurred the substances which were to be trapped may have been lost. Water vapor might have condensed from atmospheric air and the presence of carbon dioxide in the spectra show that some CO_2 from the dry ice-acetone bath used for cooling the trap, diffused into it. In view of the approach used for trapping, then, which seems not to be satisfactory although recommended in the literature[102] the mass spectra did not shed light on the nature of the interfering peak.

Examining the results in chronological order it was noted that the period when the chromatograms were obtained in which the abnormal peak made its unwanted appearance coincided with the development of a leak in the dry nitrogen supply lines to the dry box then in use for the preparation of the calcium hydride zone in the reactor. The leak was not obvious until it was observed that a static electricity effect, which charged the CaH_2 particles and made it somewhat difficult to pack them into the reactor tubing was no longer occurring due to the higher humidity inside the chamber. Packing of the reactor was then tried in other dry boxes in different laboratories in the chemistry building

but met with little success. Attempts made to obtain CaH_2 pre-ground to the adequate mesh size were unsuccessful since the main chemical suppliers sell only the coarse grade that had been obtained for the work done here. Several attempts at removing the substance causing the disturbing peak proved unfruitful.

At this stage an appraisal of the situation was made and the following conclusions were reached:

a. Drawert's experiments with BF_3-methanol were confirmed from a qualitative point of view and it is possible to make esterifications using the technique of reaction-GC alone, without a previous esterification step, as was previously stated.

b. It was found through laborious experience that the preparation of the CaH_2 zone is much too cumbersome if one has to use the method for routine analysis and no supply of finer mesh sizes is available. One to two hours have to be spent on the dry box, first grinding the hydride to size, then sieving it and finally packing the desired fraction into the reactor.

c. The reagent or water or both will interfere severely with the analysis if the CaH_2 is not properly retaining them.

d. There is a possibility of decomposition of the stationary phase material or of samples, by the

boron trifluoride-methanol catalyst, as mentioned by Schupp[(50)], if the fluoride is retained on the column.

e. Both items c and d make the CaH_2 zone a critical step in the method with all the disadvantages already mentioned.

One solution which was tried was the use of other substances in place of calcium hydride. Thus, potassium carbonate and Drierite were tried, but without success. It seems that the hydride is the only chemical that combines the useful characteristics for this particular application, namely, no retention of the esters formed, absorption of excess acid and acid catalyst, and also absorption of water, giving off hydrogen as a non-interfering reaction product.

In view of the preceding considerations and of the inordinate amount of time already spent on the BF_3-methanol approach it was decided that this line of research be put aside in favor of an esterification method using diazomethane as a reagent.

A possible solution to the problem, however, suggested itself several months after the interruption of the work on BF_3-methanol. If the main offender were water, the difficulty might be circumvented if one used a flame ionization detector which is insensitive to it. On the other hand, an investigation would still be necessary to find out the

chemical nature of the possible decomposition and/or reaction products of BF_3-methanol, with stationary phases, and their influence on the behavior of the FID by corrosion or fouling of the quartz flame tip used in some detector assemblies.

3. ESTERIFICATION EXPERIMENTS USING DIAZOMETHANE AS A REAGENT

3.01 INTRODUCTION

After the unsuccessful attempts at the use of boron trifluoride-methanol as a reagent in the direct reaction-GC esterification of fatty acids, attention was focused on a more promising reagent, diazomethane. Notwithstanding the potential dangers of its use, the reagent seemed to be suitable for experimentation with R-GC.

Diazomethane has been successfully applied to the 'external esterification' of FA as was detailed in section 1.08 of Chapter 1, the most interesting approach having been that of Schlenk and Gellerman[29]. According to these authors the rate of esterification of FA using methanol as a solvent becomes too rapid to be measured at a methanol concentration of about 2 M. The method, then, could be used with reaction-GC to further enhance esterification by (a) thorough mixing of the reagents in the reactor; (b) more flexibility in temperature control; (c) the advantages associated with reaction methods, namely, reduced sample manipulation outside the GC system to minimize volatilization and other losses. The investigation could also be conducted using various concentrations of cosolvent to determine the influence of the reactor presence, if any, on the completion of the esterification and its possible use as an analytical method.

3.02 EXPERIMENTAL

Standards. Solutions for calibration of detector response were prepared by accurately weighing about 0.2 g of the ester sought, an internal standard and a solvent into 10 or 25 ml volumetric flasks and storing the solutions in a refrigerator. The internal standards used were the following:
Experiments with octanoic acid: n-dodecane, n-tetradecane.
Experiments with myristic, palmitic and stearic acids: n-hexadecane.
Solvents used for the dilution of the acids and internal standards were either ethyl ether or benzene.
The temperature in the refrigerator was kept about 10° C.
Further details about the reagents used are given in Appendix 1, Section 2.

Acid solutions for esterification. They were prepared and stored in the same way as described for the standards. For some experiments solutions containing 10% by weight of methanol were also prepared.

Diazomethane-ether solutions. Two methods were used for the preparation of diazomethane solutions.
Method A. According to Stanley[94]. 3.1 g of KOH pellets were dissolved in 3.1 ml of distilled water in a 125 ml Erlenmeyer. The solution was cooled in an ice bath and 25 ml of ethyl ether were added. Two g of the diazomethane

precursor, N-methyl-N'-nitro-N-nitroso guanidine was weighed into a 50 ml beaker and was added in small portions over a period of several minutes to the solution in the conical flask. The flask was shaken vigorously after each addition. Throughout the preparation the appearance of a white slurry was observed and the ether layer was seen to acquire the yellow color due to diazomethane. After addition of the total amount of precursor, the ether layer was carefully decanted into another 125 ml Erlenmeyer and the flask was capped with a 'Poly-Seal' cap and stored in the freezer compartment of the refrigerator, at a temperature of -20° C. Precautions taken during the preparation were as follows: All work was carried out in a hood with the sash lowered and an opening of approximately 25 cm in height left; the room lights closest to the hood were turned off to minimize danger of photodecomposition; a plastic face mask, heavy asbestos gloves and a long sleeved lab coat were worn during all preparations. After decantation of the ether-diazomethane solution the original reaction flask was treated with a 1:1 solution of HCl or acetic acid to destroy the remainder of diazomethane.

Variation of Method A. The ether-diazomethane solution is decanted into a flask containing KOH pellets for drying. In some preparations the volume of ether was less than 25 ml for 1.5 g of precursor as recommended by Stanley and this

resulted in lower yields, the solution being less concentrated than expected. In another preparation, the solution was decanted into a conical flask containing KOH pellets and subsequently kept there but this practice is to be discouraged because apparently the solution is not so stable in the presence of KOH, some turbidity having developed. In all other experiments following this technique the diazomethane solution was dried over KOH and then transferred to a clean, dry flask.

Method B. Preparation by a distillation procedure. A combination of the procedures described by Roper and Ma[80] and deBoer and Backer was used. The distillation apparatus was an adaptation of the microdistillation apparatus used by Roper and Ma, Figure 1.01.

Procedure. 2.5 g of precursor were dissolved in ether and carbitol and placed in a pear shaped separation funnel. The total volume after complete solution of the precursor was approximately 40 ml which was above the capacity of the distillation flask, 25 ml. In subsequent preparations, a 100 ml flask was substituted for the 25 ml flask used in the first preparation tried in order to correct this inconvenience. The flask also contained a solution of 3.2 g of KOH in 3.2 ml of distilled water and 5 ml of ether. A heating plate was connected to a variable transformer and kept at its LOW control setting. Appropriate adjustment of

the transformer maintained a water bath in a 250 ml beaker at about 45° C. The level of the bath was brought to the level of the solution-ether layer in the distillation flask. The receiving vessel was a 25 ml volumetric flask placed in a Dewar containing an acetone-dry ice mixture. 16 ml of ethyl ether were measured into the receiving flask. When all components were properly connected, the water bath was brought into contact with the distillation flask and addition of the precursor solution started. The reaction was instantaneous and the diazomethane collected in the receiver until the distillation flask was ¾ full, in the case of the first experiment, or the solution in the dropping funnel was exhausted, as in all subsequent experiments.

No difficulty was experienced during the distillation and no signs of misbehaviour of the diazomethane were observed. Nevertheless all precautions cited in Method A were followed throughout the procedure which actually seemed to be safer than the previous one since no manual agitation of the reaction flask was involved, the apparatus being watched from behind a safety screen mounted on the sash of the fume hood. An improvement in the operation was added which consisted of using a magnetic stirrer-heater combination for the water bath. For this and all other distillations performed, the diazomethane solution was transferred to and kept in conical flasks taped with black plastic electrical insulating tape and fitted with 'Poly-Seal' caps.

Determination of the concentration of diazomethane ether solution in its solutions. To determine the actual concentrations of the solutions used and also to follow the useful life of the reagent, titrations were performed according to Roper and Ma[80] who modified a procedure due to Marshall and Acree. For the titrations a 5 ml reservoir burette with Teflon stopcocks was used after calibration according to directions given by Harris and Kratochvil[95]. A 0.01 M NaOH solution was prepared and standardized against potassium hydrogen phthalate following Vogel[96].

Procedure. Benzoic acid in amounts from 6.7 to 8.7 mg was weighed into three 50 ml conical flasks. About 5 ml of ethanol neutralized with 0.01 M NaOH against phenolphthalein was added to each flask. In a fume hood, diazomethane-ether solution was aspirated into a 0.1 ml serological pipet using a Pro-Pipet bulb and delivered to the conical flasks. A short effervescence and disappearance of the yellow color indicated completeness of reaction with diazomethane. The resulting solution was back-titrated with 0.01 M NaOH solution and the concentration of diazomethane calculated. For preparations according to Method A, 2 g of precursor (~147 g/mol) will give a theoretical yield of 0.57 g of diazomethane, or ~0.014 mol in 16 ml of ether solution, or a theoretical concentration (approximate) of 0.04 mg/ microliter of diazomethane.

Chromatographic Conditions. (1) The carrier gas flow rate was 60 ml/min as measured with a soap bubble flow meter. The temperature of the injector was approximately 200° C. The first column used was NPGS, neopentylglycol succinate, 10% by weight on Chromosorb W AW 60-80 mesh and packed to a 2 meter length of ¼" O.D. stainless steel tubing. Temperature of the detector block, 205° C. The column temperature was programmed from an initial temperature of 50° C to ~100° C at 2° C/min. Chromatographic Conditions, (2). The second column used was a 1.20 meter long, ¼" stainless steel tubing packed with 5% by wt. SE-30 on Chromosorb W AW 60-80 mesh. Other conditions were the same as for (1). Both columns described were prepared following the recommendations of the literature and conditioned overnight with no carrier gas flow, followed by a period of conditioning with flow until a stable baseline condition was obtained.

Calibration of detector response. (General) The standard mixtures prepared were kept either in an ice bath in a Dewar flask or in a refrigerator and were returned to the low temperature environment after use. The syringes were kept inside glass tubing of adequate diameter which served as air baths. They were in turn placed in an ice bath in a Dewar flask. This had the objective of cooling the syringes so that the sampling of the cold standard solution would be easier and also of preventing evaporation of the

solvent or components of the solution if they were drawn into a syringe at a relatively higher temperature. The syringe used at first was a Hamilton #701 Microliter syringe with a Kel-F plunger guide, but it proved to leak under conditions of use. Some injections were tried with a 0.25 ml Precision 'Gas-Lok' syringe fitted with a Teflon plunger and valve. The results improved considerably and justified the purchase of two 0.10 ml 'Gas-Lok' syringes which were used for the remainder of the investigation.

Procedure. Approximately 5-10 microliters of the standard mixture were measured and injected through the septum in the injection tee of the reactor. All injections were done this way through the reactor. The column temperature was programmed after the solvent peak was eluted but no effort was made to keep the same initial temperature time interval before start of programming. Chromatographic conditions (from now on to referred to as C.C. 1 or 2) were either 1 or 2 depending on the experiments run as specified in the text. Reactor temperature was 50° C or kept above the boiling point of the components of the standard mixture as noted in relation to specific series of experiments in other parts of the text. The peak areas were obtained by electronic integration and the response factor calculated as shown below.

Calculation of response factors. The method of quantitative

analysis chosen for this investigation was the internal standard method. Its advantages are described in the literature by Harris, Habgood[97], and by Littlewood[98] among others. Instead of plotting a calibration curve, the determination of a single response factor which is the slope of the curve, was chosen. It was assumed that the system was linear for the range of concentrations to be studied which for the TC cell detector is true within wide limits. The response factor is determined by equation 3.01:

$$F_s = \frac{W_i \cdot A_s}{W_s \cdot A_i} \tag{3.01}$$

where F_s = response factor

W_i = weight, added, of internal standard

W_s = weight, added, of sample

A_s = area obtained, sample

A_i = area obtained, internal standard

The average of all factors calculated from a number of chromatograms is taken as the response factor for the calculation of esterification yields as shown further below.

Esterification experiments. Two types of esterification experiments were performed, one in which the esterification solution was composed of a fatty acid, internal standard and solvent only, another in which 10% methanol was added to the mixture. The same problem with sampling syringes

was found as described under Standardization, and the best solution available was the use of the 'Gas-Lok' syringes.

Injection Procedures. Different procedures were used for the esterification experiments. They were variations of a general method and had different purposes as seen under the Discussion.

A. 'Slug Injection'. The calculated volume of excess diazomethane solution and a volume of the acid solution to be esterified were measured into a syringe, an air slug left between the two solutions in the barrel. The syringe charge was then injected rapidly through the reactor septum. After elution of the solvent peak the column temperature was programmed as described for the standard mixture.

B. 'Stopped-flow Injection'. Here the apparatus was used in Configuration C. The 3-way valve was closed, an injection made using the same sampling technique as for a slug injection and a delay of 30 seconds was made before re-establishing carrier gas flow through the reactor, at a temperature of 50° C. With this technique the delay time could be varied within reasonable limits for an investigation of its effects.

C. 'Double-syringe Injection'. Two identical syringes are used, one containing the acid mixture, the other the diazomethane solution. During injection, the two needles pierce the septum simultaneously but the injection of the liquids

can be either simultaneous or lagged, fast or slow, the acid solution being injected first, followed by the diazomethane solution.

Reactor temperature. Most esterification experiments were made with the reactor operated isothermally at a low (50° C) or higher temperature (190-250° C) but another technique was also used, that of raising the reactor temperature rapidly with a Variac, e.g., from 50° C to 190° C or 200° C a few seconds after injection.

Calculations. After GC elution of the esterified mixture the peak areas found for the ester and internal standard are related to the ester yield in the following way: The weight of the ester obtained in the esterification is given by Equation 3.02:

$$W_s = \frac{A_s \cdot W_i}{A_i \cdot F_s} \qquad (3.02)$$

The symbols have the same significance as in Equation 3.01. The theoretical yield is calculated in the usual manner from the molecular weights of FA and FAME involved:

$$\left(\frac{\text{Mol. wt. ester}}{\text{Mol. wt. F.A.}}\right). \ \text{Wt. acid, taken} = W_s\text{, theoretical} \qquad (3.03)$$

The practical or actual yield is, then,

$$\frac{W_s}{W_{s,th}} \cdot 100 = \%\ \text{yield or}\ \%\ \text{conversion} \qquad (3.04)$$

Preparation of Pure n-Hexadecane by Preparative GC.

General. Due to unavailability of pure n-hexadecane which was found to be a suitable internal standard for C_{12}-C_{16} esters, it was necessary to purify the Eastman P 3389 grade n-hexadecane by preparative gas chromatography.

The apparatus used was an Aerograph Autoprep A-700, located in Lab. 306, used by permission of those responsible for it. Initially, the injection port of the instrument was cleaned using a pipe cleaner and acetone, chloroform and benzene in successive applications until it was free of carbonaceous residua from previous injections. A 20-ft., 3/8" O.D. aluminum column packed with SE-30, 30% on Chromosorb W 30/60 mesh was borrowed from Dr. W. Ayer laboratory and used in the operation. Instrument conditions were as follows:

Injector temperature = 200° C

Detector temperature ~225° C

Column temperature, initial ~80° C

Column temperature, final ~250° C.

At 30 psig the reference flow rate (with helium as the carrier gas) was measured as 16 ml/min. The sensing side flow rate was approximately 200 ml/min. Cell current was 150 mA and the recorder chart speed, 0.5 in/min.

The automatic fraction collector on the instrument was inoperative so the fractions had to be collected manually, but since only one peak was being collected, that did not

cause difficulties. Programming of the temperature was done as recommended in the instrument manual, by step-programming, up to 250° C.

A preliminary run was made and a small volume of the peak suspected to be n-hexadecane was collected for confirmation of identification by NMR analysis. Results obtained were

$^{H}CH_2$ -- 12.1 units

$^{H}CH_3$ -- 2.5 units. Assuming a molecular formula $C_{16}H_{34}$, one has 34 H/14.6 units or ~2.3 H/unit, or H_{CH_2} = 28; H_{CH_3} = 6 or total H = 34, which confirms $C_{16}H_{34}$ for the peak trapped.

Procedure. After stabilization of initial conditions on the instrument a 0.5 ml sample was measured into a 500 microliter Hamilton #1750 GasTight syringe fitted with a 6-inch needle for on-column injection. The sample was discharged slow and continuously and after withdrawal of the needle the oven temperature control was manipulated to begin the step-programming. The fraction desired, n-hexadecane, was collected using an Aerograph 1 ml collector bottle (Model 66-023). Its inlet tube was reduced to ~2 mm O.D. to fit the septum on the outlet of the instrument. The collector was placed in a water bath, contained in a Dewar flask, at room temperature. The first attempt at sample

collection was not successful because of aerosol formation and considerable sample loss ensued. A wad of glass wool was then placed loosely inside the neck of the outlet arm of the collector and it was observed that the aerosol was not forming as soon as enough liquid had been condensed on the glass wool. After collection the bottles were placed in a bench centrifuge and most of the liquid retained on the glass wool was recovered. In all the experiments collection was started a few seconds after the leading elution edge of the peak to ensure purity of the fraction collected.

Purity check. The fraction collected was eluted in the reaction-GC instrument and also in a 5750 Hewlett-Packard gas chromatograph located in Lab. 306. No impurities were detected. Some TLC plates were also used to check purity of the compound as contrasted to that of the practical grade. Results were confirmatory of the purity of the n-hexadecane obtained.

3.03 RESULTS AND DISCUSSION

(1) Experiments with octanoic acid. Calibration of Detector Response. The composition of the first standard solution prepared was:

	Wt. added	Wt.%	Mol%
Octanoic acid methyl ester	0.2064	50.0	52.0
n-Dodecane (I. Std.)	0.2061	49.9	48.0

Solvent used was ethyl ether, 17.3174 g. This makes the solution 1.16 wt % in ester and in int. std.

The results of several determinations of the response factor for that mixture are shown in Table 3.01, which presents two sets of results using different syringes. The results obtained can be summarized as follows:

1st Set:	Average, F_s	0.914_3
	Standard deviation	0.04
	Relative std. deviation	4.4%
2nd Set:	Average, F_s	0.917_3
	Standard deviation	0.03
	Relative std. deviation	3.0%

The relative standard deviation shows a large improvement of the second set of results over the first, which determined the use of the Pressure Lok gas syringe in all subsequent experiments. Two determinations were made 9 days later, on the same mixture, with the objective of detecting changes in the response factor resulting from changes in instrumental conditions. Results obtained are shown in Table 3.01-C.

In order to verify any significant difference from the mean the "t" test was applied. t = 0.1098 which is a value many times less than the values at 99% level(99). These results are helpful in showing that changes in either experiment conditions or in the standard solution due to storage

TABLE 3.01 DATA FOR CALIBRATION OF DETECTOR RESPONSE FOR METHYL OCTANOATE USING n-DODECANE AS INTERNAL STANDARD

Area, ester (A_s)	Area, internal standard (A_i)	Ratio, (A_s/A_i)	Response Factor, F_s	Deviation from Mean
(Arbitrary integrator counts)				
		A		
4260	4616	0.922_8[b]	0.921_5	$+0.007_3$
10063	10918	0.921_7	0.920_3	$+0.006_1$
12420	13382	0.928_1	0.926_8	$+0.012_5$
11544	12702	0.908_8	0.907_5	-0.006_8
10220	10998	0.929_3	0.927_9	$+0.013_6$
9699	10627	0.912_7	0.911_4	-0.002_9
8093	8507	0.951_3	0.949_9	$+0.035_7$
9328	10639	0.876_8	0.875_5	-0.038_8
11475	12901	0.889_5	0.888_2	-0.026_1
9696	9687	1.000_9	0.999_5	$+0.085_2$
10330	12451	0.829_7	0.828_4	-0.085_8

A (Results obtained with Hamilton 701 syringe).

		B		
62521	66492	0.970_4	0.968_9	$+0.051_6$
17976	20051	0.896_6	0.895_2	-0.022_1
21054	23047	0.913_5	0.912_2	-0.005_1
25067	27269	0.919_2	0.917_9	-0.000_6
25314	27246	0.929_1	0.927_7	$+0.010_4$
24959	28262	0.883_1	0.881_8	-0.035_5

B (Results obtained with a Precision Sampling Pressure-Lok gas syringe).

TABLE 3.01 (continued)

Area, ester (A_s) (Arbitrary integrator counts)	Area, internal standard (A_i)	Ratio, (A_s/A_i)	Response Factor, F_s	Deviation from Mean
		C		
26909	24881	0.924_6	0.923_3	$+0.006_0$[a]
69161	61830	0.894_0	0.892_7	$+0.024_7$[a]

C Random determination of response factor for octanoic acid methyl ester solution.

a Deviation from previous mean.

b Values are not reliable to ±1 in the last digit, if it is shown as a subscript in this and other tables.

or chemical decomposition were not significant.

Esterification experiments with octanoic acid. Composition of solutions used. Solution 1, octanoic acid, 0.0812 g, (or 1.1 wt.%, 0.056 mol%); n-dodecane, 0.0723 g (1.0 wt.%, 0.042 mol %). Solution 2, 0.0864 g, octanoic acid (1.2 wt. %, 0.059 mol %); 0.0823 g n-dodecane (1.2% wt. %, 0.048 mol %).

Solution 1 was used in experiments 1-16, solution 2 for experiments 17, 18, the results of which are given in Table 3.02. From the results obtained some points are apparent:

a) no great advantage is shown by using different injection techniques;

b) no improvement is shown by the use of 10% methanol in

TABLE 3.02 RECOVERY OF METHYL OCTANOATE FROM ESTERIFICATION EXPERIMENTS OF OCTANOIC ACID[a]

Exper. #	Integrator area counts: ester	I. std.	Ester added, grams	Ester found, grams	Yield, %	Reactor temperature, °C	Residence time, approx., in reactor (seconds)	Volumes of sample and diazo-solutions injected
1	29514	33581	0.0892	0.0647	73.0	50	18	0.02μl-0.1μl
2	16020	29346		0.0402	45.0	50	18	0.02μl-0.1μl
3	28360	42254		0.1174	132.0	50	18	2.5μl-4μl
5	18341	16068		0.0899	100.8	50	30	2.5μl-4μl
7	25598	31068		0.0624	70.0	190	18	2.5μl-4μl
8	22913	28406		0.0636	71.0	190	18	2.5μl-4μl
9	4216	15878		0.0209	24.0	190	18	2.5μl-4μl
10	20033	34665		0.0455	51.0	190	18	6μl-0.06ml
11	13070	19805		0.0520	58.0	190	18	6μl-0.06ml
12	4664	10373		0.0354	40.0	50	18	10-40μl
13	18785	38099		0.0389	44.0	50	20	10-40μl
14	17175	30350		0.0446	50.0	50	28	10-40μl
15	14969	34714		0.0339	38.0	50	33	10-40μl
16	7479	12033		0.0489	55.0	50	18	10-40μl
17	13653	19948		0.0539	61.0	50	18	8μl-70μl
18	11017	20720		0.0419	47.0	50	48	9μl-90μl

a Column temperature: initial, 50°, final 100°C, programming rate: 2°C/min;
response factor in calculations: 0.981_4 (cf. Table 3.01);
flow rate: 50 ml/min; instrument configuration: "B"; internal standard: n-dodecane;
one syringe injection for #1-#10; 2 syringes for the remainder of experiments;
column: NPGS, 10% by wt. on Chromosorb W AW 60-80 mesh.;
methanol added, 10% by wt.

'Ester added' in this and following Tables means theoretical yield of ester expected from the esterification of the acid actually added to the esterification solution.

the esterification solution nor was the drying of the diazo solution over KOH, done for some experiments, helpful;

c) experiments 12-15 are of doubtful value. They increased the residence time by the technique of 'stopped-flow' but the conversions are low. The randomness of the % yields, however, does not point at any generalization at this stage.

It is to be noted, however, that estimating the residence time in the reactor as approximately 18 seconds for a carrier gas flow rate of 50 ml (approximately) and assuming all reaction between the diazomethane solution and acid to occur during this residence time, the preliminary results of experiments 1 to 11 were taken as indicative of the potentiality of the technique of reaction-GC using diazomethane as a reagent if one compares the results with those of Schlenk and Gellerman who accomplished esterifications in 10 and 30 minutes even using 10% methanol in the ether solution which they claim to make the esterification too rapid to be measured. Since some esterification experiments in the Table resulted in yields above 100%, the possibility was considered that previous injections had left unesterified sample in the reactor and/or column and a 'memory effect' was producing the abnormal results. An injection of pure diazomethane was made to find out if that was happening. The resultant chromatogram is shown in Figure 3.01. It is observed that peaks for both the standard

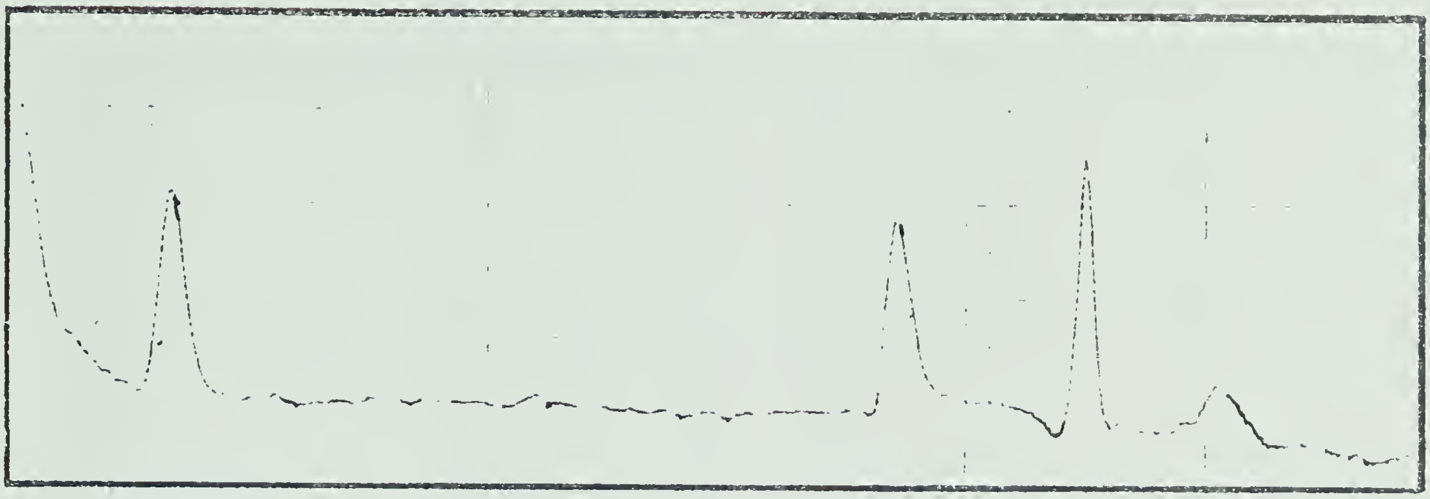


Figure 3.01 A diagram illustrating the occurrence of a 'memory effect'.

and the ester appeared on the chromatogram confirming the suspicion above. Unidentified peaks other than that due to the solvent are also present. Another possibility was that the column liquid, being a polyester, might be undergoing interesterification with residual diazomethane but this was not proved conclusively.

Problem with the internal standard. In some of the later chromatograms there appeared a slight shoulder on the leading edge of the internal standard peak, which later became a distinct overlapping peak. Figure 3.02a shows the chromatogram obtained in the first esterification performed with diazomethane and b the chromatogram obtained in experiment 13, where the former has all the characteristics of a 'normal' peak, the latter showing the unidentified

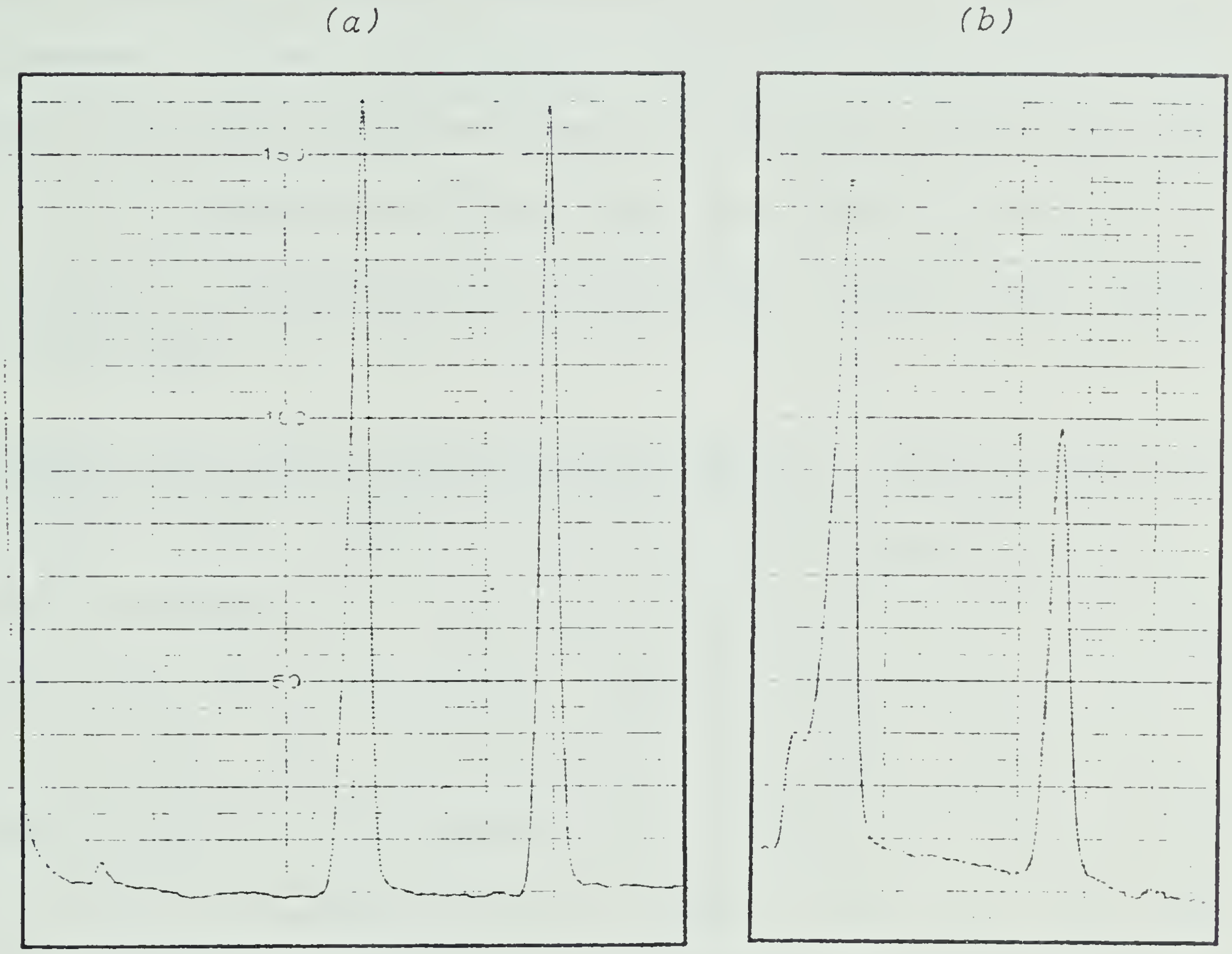


Figure 3.02 (a) First esterification performed showing the regular shapes of the peaks; (b) the overlapping peak which appeared later.

overlapping peak. In those chromatograms where the overlap is not resolved enough to trip the integrator, the two areas are recorded as one and this affects the percentage yield seriously, resulting in lower yields than if the overlap were not present.

To circumvent the present problem it was thought that the adoption of a new internal standard was necessary. Experiments with some compounds decided in favor of n-tetradecane,

which eluted at a higher retention temperature than methyl octanoate. It was anticipated that the interfering peak would not cause problems with n-tetradecane and examination of the chromatograms did not show any peaks appearing in the region of elution of n-tetradecane, giving more support to this idea.

Problems with the diazomethane solution. For the set of experiments 12-15 a fresh solution of diazomethane was prepared, following the variation of Method "A", but its color, when drawn into the syringe, appeared paler than the previous one. At this time, no control had been made of the actual concentration of diazomethane by titration, in the solutions used, so it was judged from its aspect alone that the lower yields obtained were due to a deficiency in diazomethane concentration. The only possible difference in the preparation procedure was the use of a smaller quantity of ethyl ether as a solvent than recommended, which might have resulted in a lower extraction yield. At this stage a recommendation by Roper and Ma[80] concerning the use of diazomethane solutions decanted from water was causing growing concern but the alternative preparation of diazomethane by distillation was being kept in mind as a last resort due to the alarming warnings found in the literature concerning its greater potential danger to cause violent explosions.

Problems with equipment. For all experiments cited in

Table 3.01, the GC instrument used was configuration B (III). Problems arose with peak broadening, and to remedy this the reactor temperature was raised from 50° C to 200° C. There was, of course, a sensible improvement in peak shape for n-dodecane. The reactor, however, could not be operated at such a high temperature from the beginning of the injection because that would lead to a faster thermolysis of diazomethane. Another solution was to abandon configuration B and use a different means for a faster heating/cooling cycle than was possible with the large brass block. This resulted in the design and adoption of Configuration C (described also in Appendix III). Upon dismantling the previous reactor for effecting the changes it was observed that the quartz wool packing on the extremity closest to the 3-way valve was discolored, showing a light brown color. (Figure 3.03).

Figure 3.03 Fragment of quartz wool. The discoloration was evidence of pyrolysis caused by a 'hot spot' in the connecting tubing.

Since the heating was accomplished by tapes to avoid sample condensation in the lines, a hot spot had probably formed at that location due to the impossibility of making the

temperature uniform throughout when using the tapes in direct contact with the metal tubing and was causing pyrolysis of sample components.

The change in reactor design alone did not improve the peak shapes since the geometry of the inter-connecting tubing was unchanged. The next improvement was thermal lagging with glass wool underneath the oven platform which resulted in the use of lower voltages in the Variacs for the same temperature. The lagging improved results somewhat as far as peak shapes were concerned.

Problems with column partitioning liquid. As soon as preliminary esterification runs were made with n-tetradecane as internal standard the chromatograms showed a small but definite shoulder on the leading edge of its peak. This overlapping peak was not present in the trial runs with pure n-tetradecane so the problem was not solved although it was not easy at all to explain the interference, on the basis of previous experience. To find out if the column was adding peaks of its own, through aging or decomposition, a new NPGS column packed in a glass coil was used in some experiments with injection of pure diazomethane solution. The results show some peaks as previously found, which can be seen in Figure 3.04.

A fresh approach to the problem would be to change the partition liquid in the column. To this end a 5% by wt. SE-30

on Chromosorb W AW 60/80 mesh column was prepared. A sample of diazomethane solution in ether was injected and it was observed that several peaks appeared on the chromatogram but all had much lower retention times than those on NPGS. (Figure 3.05). The column was isothermal at 30° C. The column was then sylanized by injection of 50 microliters of "Silyl 8" column conditioner (Pierce Chemical Co.) at a column temperature of 170° C. The treatment improved the shapes of methanol peaks although results were not dramatic. Three esterification experiments were run with the system in the present condition. The results obtained were 57, 63 and 49%. No improvement occurred in percentage yield.

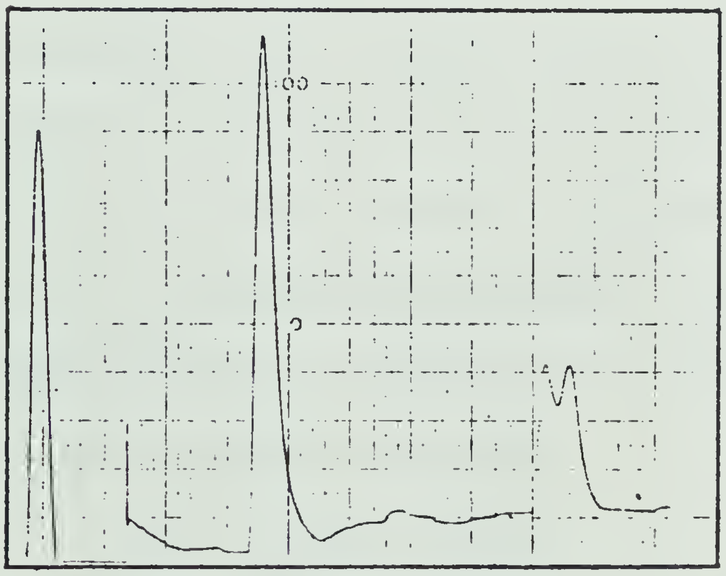


Figure 3.04 Unidentified peaks eluted upon injection of diazomethane-ether solution (NPGS column).

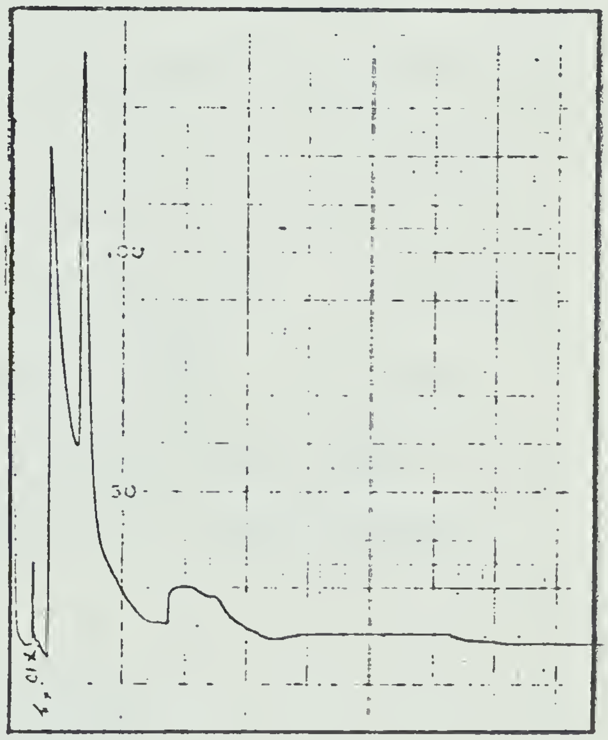


Figure 3.05 Unidentified peaks from injection of diazomethane on an SE-30 column, at 30° C.

Change in system configuration. At this stage, it was decided to have all connections to the column, reactor and detector as short as possible and uniformly heated without tapes. The results was Configuration "D" (described in Appendix III) with which new experiments were tried.

Experiments with octanoic acid and n-tetradecane as internal standard. A second standard solution was prepared, taking 0.2999 g of methyl octanoate and 0.2449 g of n-tetradecane. Benzene was used as solvent, 13.5084 g taken. This makes the composition of the solution 2.2 wt. % ester, 1.8 wt. % tetradecane. The results obtained in several calibration runs are given in Table 3.03. Two of the results, 25 A and 25 G were examined by application of the "Q" test. The Q value at the 90% level for F_s including results A to J is 37, which is less than 0.41 for N = 10 (Table 4-18, Harris & Kratochvil, page 206)[95]. If the value for 25 A is not included, the new value for Q is 0.74, which is greater than 0.41. It was then found justifiable to discard both 25 A and 25 G. The set of determinations now give

F_s, average value	0.905_1
Standard deviation	0.03
Relative stand. deviation	3.2%

The same order of magnitude for the relative standard deviation was obtained for the two previous sets of determinations.

TABLE 3.03 DATA FOR CALIBRATION OF DETECTOR RESPONSE FOR METHYL OCTANOATE USING n-TETRADECANE AS INTERNAL STANDARD

Expt. # 25	Area, ester (A_s) (Arbitrary integrator counts)	Area, I. std. (A_i) (Arbitrary integrator counts)	Ratio (A_s/A_i)	Response factor F_s	Deviation from mean
A	15353	17315	0.886_7	0.724_1	a
B	46051	42901	1.073_4	0.876_6	-0.028_6
C	33529	29823	1.124_3	0.918_1	$+0.012_9$
D	62947	54974	1.145_0	0.935_0	$+0.029_9$
E	59122	52178	1.133_1	0.925_3	$+0.020_1$
F	55754	51466	1.083_3	0.884_6	-0.020_5
G	30915	42077	0.734_7	0.599_9	a
H	56370	54240	1.039_3	0.848_7	-0.056_5
I	51489	46982	1.095_9	0.894_9	-0.010_2
J	39840	34929	1.140_6	0.931_4	$+0.026_3$
K	42919	39274	1.092_8	0.892_4	-0.012_8
L	42844	37502	1.142_5	0.932_9	$+0.027_8$
M	42690	38027	1.122_6	0.916_7	+0.0116
61[b]	192887	175926	1.096_4	0.895_3	-0.009_9
61A[b]	99510	90466	1.099_9	0.898_2	-0.013_0

a Results discarded on the basis of the "Q" test. (See text).

b Expts. 61 and 61A were determinations of F_s made on this solution 26 days after its preparation. The results are within the limits previously found.

<u>Esterification experiments with octanoic acid and n-tetradecane as internal standard</u>. The results obtained in a set of experiments are given in Table 3.04. They are

TABLE 3.04 SECOND SET OF ESTERIFICATION EXPERIMENTS WITH OCTANOIC ACID[g]

Expt. Designation	Integrator counts		Ester found	Yield or % Conv.	Reactor temper; °C
	Ester	I. Std.			
48	50254	47363	0.1014	68	50° 230°C
50	68381	54626	0.1196	80	25° 200°
51[a]	29437	19631	0.1433	96	200°
52[b]	31162	20685	0.1439_6	96	190°C
53[c]	24916	14658	0.1624	108	190°
54[d]	78583	58245	0.1289	86	190°
55[d]	71158	57258	0.1187	79	190°
56[d]	67284	43886	0.1465	98	190°
58[e]	4895	14518	0.0322	22	
59[f]	20217	15230	0.1269	85	200°
59A[f]	13929	10607	0.1255	84	200°
59B[f]	18544	13959	0.1269	85	200°
60	13896	9882	0.1344	90	60°

a External esterification: 0.5 ml diazo sol. added to 10 μl ester sol. in a conical bottom rubber capped vial; sample taken after 3 minutes.

b Same as 'a', but conical flask cooled to 20° C in water bath; 10 μl sample added, stopwatch started, 1st sample withdrawn after exactly 5 minutes.

c idem (b) sample withdrawn after 1 hr. 20 min. 12 μl injected. Most of sample had evaporated, samples not representative.

d All performed exactly the same way for repeatability.

e Anhydrous methanol added to a vol. of diazomethane sol.

TABLE 3.04 (Continued)

which was then used.

f All performed exactly the same way for repeatability; methanol (0.1 ml) added to 1 ml diazo sol. at 0°C and resulting solution used.

g Flow rate: 50 ml/min;
instrument configuration: "D";
response factor used in calculations: 0.905_2;
internal standard: n-tetradecane;
injections: "double-syringes" method;
column: SE-30 5% by wt. on Chromosorb W AW, 60-80 mesh;
ester added: 0.1494 g;
residence time in reactor 18 seconds, except in Expt. 50, 23 seconds;
volume of sample injected; 10 µl; of diazomethane, 100 µl.

---

still not satisfactory. Since all the possible alternatives have been tried to increase the yields there remained only the attempt at using diazomethane solution prepared by distillation (method "B" in the Experimental Section). The preparation did not present problems or emergencies of any kind. Unfortunately the concentration of diazomethane was not determined by titration after its preparation. A sample was injected through the reactor at room temperature and another at 200° C. The difference between the two chromatograms is seen in Figure 3.06, which should also be compared to Figure 3.05. The difference between the two is striking, the distilled solution giving a much "cleaner" chromatogram.

Esterification experiments were tried immediately with octanoic acid. The results are collected in Table 3.05.

(a)

(b)

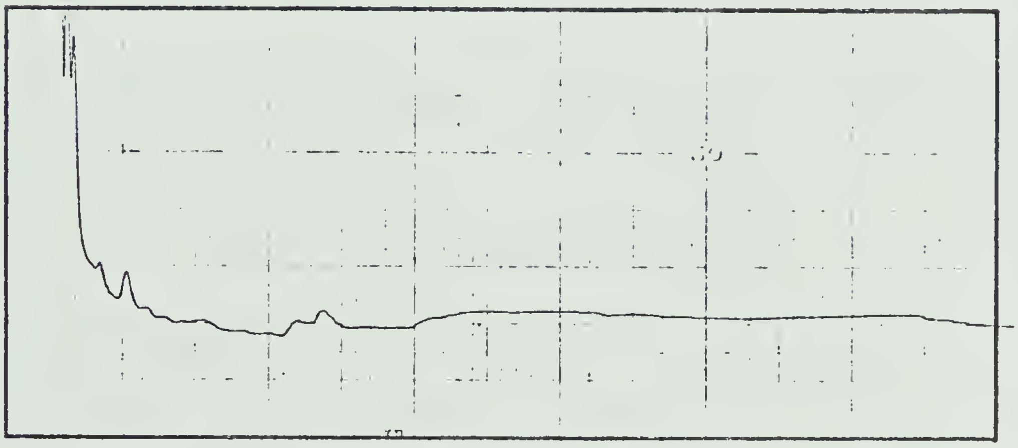


Figure 3.06 Chromatograms obtained upon injection of distilled diazomethane solution: (a) reactor at room temperature; (b) reactor at 200° C. (Column programmed from 40° to 100 C in both cases).

TABLE 3.05 EXPTS. WITH FRESHLY DISTILLED DIAZOMETHANE IN ETHER SOLUTION – ESTER. OF OCTANOIC ACID[d]

Expt. designation	Integr. counts Ester,	I. Std.	Ester Added g	Ester found g	Yield or % conversion
65[a]	74817	46610	0.1494	0.1534	102.7
66[b]	81378	52085	0.1494	0.1493	99.9
67[c]	0	0	0	0	0
68[b]	49316	30843	0.1494	0.1528	102.3
69[b]	97435	60730	0.1494	0.1533	102.6

TABLE 3.05 (Continued)

a, b Expts. run on subsequent days.

c "Scavenger run", diazomethane sol. injected to find out if any unesterified acid from previous run had been retained in reactor and/or column.

d reactor: isothermally held at 60°C; residence time, ~18 seconds in all experiments; volume relation of diazomethane solution to sample: ~10:1 in all experiments.

They are self-explanatory. Since the esterification solution was exactly the same as for Table 3.04, the results obtained, all denoting completeness of the esterification, can be attributed to the merits of the distilled diazomethane solution relative to those of the solutions previously obtained from decantation from water in the reaction vessel, and perhaps also to the action of methanol as a cosolvent. The average ester found in the experiments in Table 3.05 was 0.1522; std. dev., 0.002; rel. std. dev., 1.27%.

The composition of the solution used for esterification was octanoic acid, 0.1362 g (1.85 wt. %; 0.094 mol%); n-tetradecane, 0.0865 g (1.18 wt. %; 0.044 mol %).

2. <u>Experiments with myristic, palmitic and stearic acids:</u>

After the accomplishment of the successful esterifica-

tion with octanoic acid it was decided that the next step in the investigation would be performing experiments with higher molecular weight acids, in the C_{10} - C_{20} range such as myristic, palmitic and stearic acids.

Choice of internal standard. Hexaethylbenzene (B. P. 298.3°C) was tried but its retention temp. coincided with that of methyl myristate. Practical grade n-hexadecane was the next choice and it eluted before and near the methyl myristate peak. Prior to use it was purified by preparative GC, as described in Section 3.2 (Experimental).

Calibration of detector response. A standard solution was prepared with C_6H_6 as solvent. The following weights were taken:

methyl myristate, 0.2162 g (1.0% by wt.)
methyl palmitate, 0.3485 g (1.7% by wt.)
methyl stearate, 0.2183 g (1.1% by wt.)
Int. Std., 0.2923 g (1.4% by wt.)

Tables 3.06A, B and C show the calibration data obtained from this standard solution for each of the esters sought. The standard deviation and relative standard deviation for the results show significant improvement over those obtained for methyl octanoate (Table 3.01).

TABLE 3.06 DATA FOR CALIBRATION OF DETECTOR RESPONSE FOR C_{14}^{-}, C_{16}^{-} and C_{18}^{-} METHYL ESTERS

Expt. No. 85	Ester Integrator Counts (Areas)	I. Std.	Areas Ratio $(\frac{A_s}{A_i})$	Response (F_s) Factor	Deviation from mean
			A		
M	42349	60528	0.699_6	0.945_9	-0.012_7
N	33940	49382	0.687_3	0.929_2	$+0.004_0$
O	34658	51208	0.676_8	0.915_0	$+0.018_2$
P	17074	24544	0.695_7	0.940_6	-0.007_4
R	25526	36580	0.697_8	0.943_4	-0.010_2
S	35607	52033	0.684_3	0.925_2	$+0.008_0$
			B		
M	66441	60528	1.097_6	0.920_6	$+0.000_2$
N	53924	49382	1.091_9	0.915_8	$+0.005_0$
O	55594	51208	1.085_6	0.910_5	$+0.010_3$
P	27492	24544	1.120_1	0.939_4	-0.018_6
R	40641	36580	1.111_0	0.931_8	-0.011_0
S	56244	52033	1.080_9	0.906_6	$+0.014_2$
			C		
M	37983	60528	0.627_5	0.840_2	$+0.004_2$
N	31834	49382	0.644_7	0.863_3	-0.018_9
O	32246	51208	0.629_7	0.843_2	$+0.001_2$
P	15365	24544	0.626_0	0.838_2	$+0.006_2$
R	23166	36580	0.633_3	0.847_9	-0.003_5
S	32406	52033	0.622_7	0.833_8	$+0.010_6$

A, data for C_{14}^{-}; B, for C_{16}^{-}; C, for C_{18}^{-}.

For methyl myristate, mean 0.933_2; std. dev. 0.012; rel. std. dev. 1.3%. For methyl palmitate, mean 0.920_8; std. dev. 0.013; rel. std. dev. 1.4%. For methyl stearate, mean 0.844_4; std. dev. 0.010; rel. std. dev. 1.2%.

<u>Esterification experiments with $\bar{C}_{14}$, $\bar{C}_{16}$, $\bar{C}_{18}$ acids.</u> The solution shown in Table 3.07 was used in the esterification experiments with high molecular weight saturated fatty acids:

TABLE 3.07 COMPOSITION OF ESTERIFICATION SOLUTION

Compound	wt. taken g	mol. wt., g/mol	mols taken	mol %	wt. %
C_6H_6	7.4076	78.11	0.0948	75.9_6	81.5_8
CH_3OH	0.8650	32.04	0.0269	21.5_5	9.5_3
$C_{16}H_{34}$	0.2505	266.45	0.0009_4	0.7_5	2.7_6
C_{14}-acid	0.1575	228.36	0.0006_9	0.5_5	1.7_3
C_{16}-acid	0.2001	256.42	0.0007_8	0.6_2	2.2_0
C_{18}-acid	0.1999	284.47	0.0007_0	0.5_6	2.2_0

The theoretical yields of methyl esters calculated from the composition of the solution given in Table 2.07, assuming 100% conversion are:

methyl myristate, 0.1672g

methyl palmitate, 0.2110g

methyl stearate, 0.2098g

These values were used to calculate the actual yields. The results obtained from several esterification experiments are presented in Table 3.08.

TABLE 3.08 RECOVERY OF METHYL ESTERS OF HIGH MOLECULAR WEIGHT FATTY ACIDS[a]

Exper. #	Integrator counts for Areas				Ester found, g			Yields, %		
	Me Myristate(1)	Me Palmitate(2)	Me Stearate(3)	I. Std.	(1)	(2)	(3)	(1)	(2)	(3)
87	9572	10047	6856	17588	0.1461	0.1554	0.1157	87.4	73.7	55.1
88	54685	50909	44168	72819	0.2016	0.1902	0.1799	120.5	90.2	85.8
89	45603	55721	51028	66376	0.1844	0.2284	0.2281	110.3	108.3	108.7
90	21616	21779	21616	44021	0.1318	0.1346	0.1457	78.8	63.8	69.5
92	70729	73662	51108	120264	0.1579	0.1666	0.1261	94.4	78.9	60.1
93	24275	28550	25712	46150	0.1412	0.1701	0.1653	84.4	80.6	78.8
93[b]	2.51	3.04	2.85	4.89	0.1378	0.1692	0.1729	82.4	80.2	82.4
94	37698	41204	24301	57183	0.1769	0.1961	0.1261	105.8	92.9	60.1
96	37275	49584	44321	58126	0.1721	0.2321	0.2262	102.9	110.0	107.8
97	16701	25820	22168	29942	0.1497	0.2346	0.2197	89.5	111.2	104.7
98	36048	48367	48023	58256	0.1661	0.2259	0.2446	99.3	107.1	116.6
101	46713	60392	d	79762	0.1572	0.2060	d	94.0	97.6	d
102	70500	87422	85832	130518	0.1449	0.1823	0.1951	86.6	86.4	93.0
103	35672	43711	42731	60804	0.1575	0.1956	0.2085	94.2	92.7	99.4
105	55465	57615	28317	92713	0.1606	0.1691	0.0906	96.0	80.1	43.2
106[c]	49	3046	17416	92713	0.1607	0.1780	0.1464	96.1	89.4	69.8
107	51347	54367	38545	84130	0.1638	0.1758	0.0961	97.9	83.3	45.8
111	79363	97616	93013	122301	0.1742	0.2172	0.2256	104.2	102.9	107.5
113	80013	132055	123651	134176	0.1601	0.2678	0.2734	95.7	126.9	130.3
115	101332	115254	102310	197338	0.1378	0.1589	0.1538	82.4	75.3	73.3
116[c]	+ 10447	+ 42896	+ 31782	231	0.1519	0.2178	0.2014	90.8	103.2	95.9

a. Column temp.: Initial, 60° C, final, 210° C

Instrum. config. used: D

Response factors used: 0.933_2, 0.920_8, 0.844_4 (for Me myr., Me. palm., Me stear.)

flow rates: expts. 87 to 113, 50 ml/min; expts. 114 to final, 20 ml/min.

residence times: expts. 87 to 113, 18 seconds, expts. 114 to final, 42 seconds

reactor temperature: before injection, ~50° C; raised to ~230° C 20 seconds after injections for all experiments.

b. Areas calculated graphically for rough check of integrator.

c. "Recovery" runs.

d. Integrator counts lost for this peak.

The esterification experiments run with the three higher molecular weight acids show wide discrepancies in the yields obtained. Some anomalies observed on the chromatograms were studied as possible clues to the discrepancies, e.g.:

(1) shoulders were noticed on leading or trailing edges, or both, of the internal standard peak, Figure 3.07.

(a)

(b)

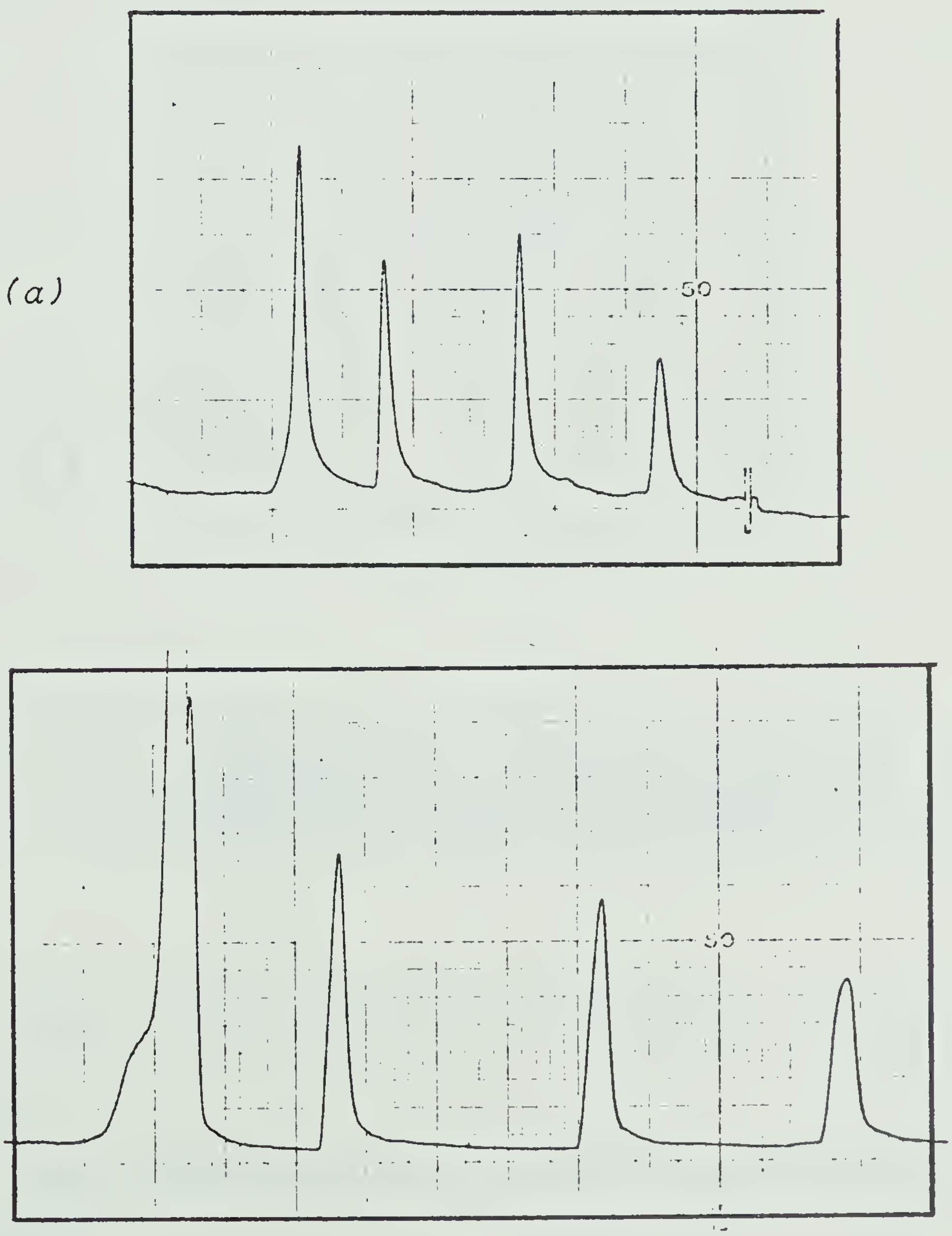


Figure 3.07 Overlapping peak on the leading edge of the int. std. peak. (a) barely noticeable; (b) appearing as a distinct shoulder.

(2) the shape of the methyl stearate peak is not sharp, like the others, but of an unusual type, as if two peaks were overlapping close to their apexes. (Figure 3.08).

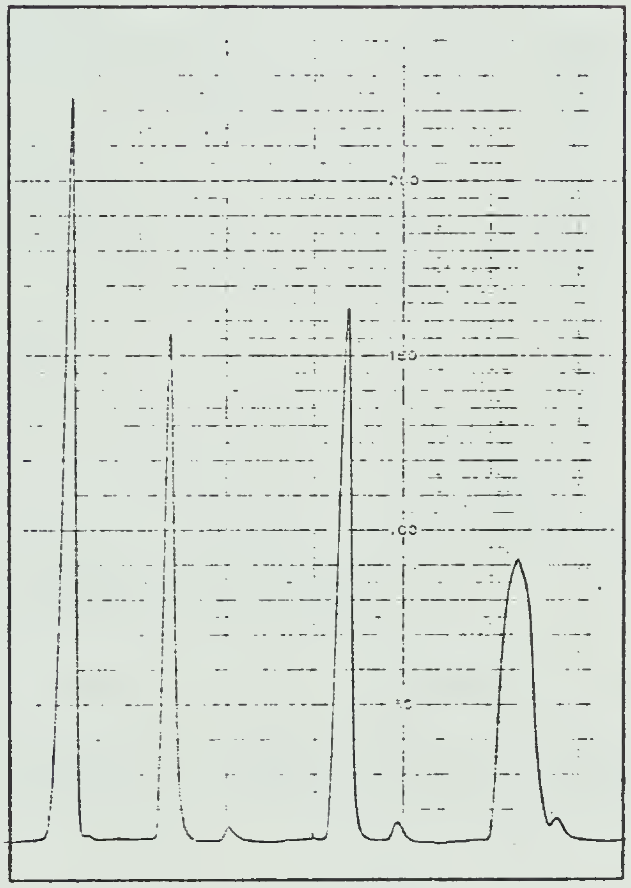


Figure 3.08 *From left to right, the tallest peaks are: n-hexadecane, methyl-myristate, methyl palmitate and the unusually shaped methyl stearate. The small peaks are unidentified.*

(3) peaks would sometimes tail badly, as shown in Figure 3.09.

(4) small peaks appeared between the main peaks. (Figure 3.08).

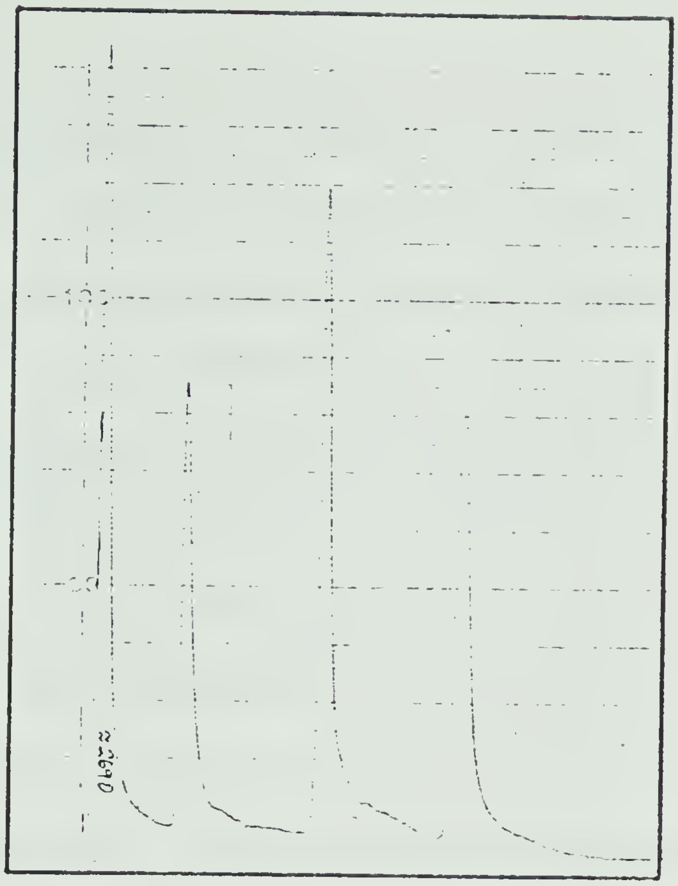
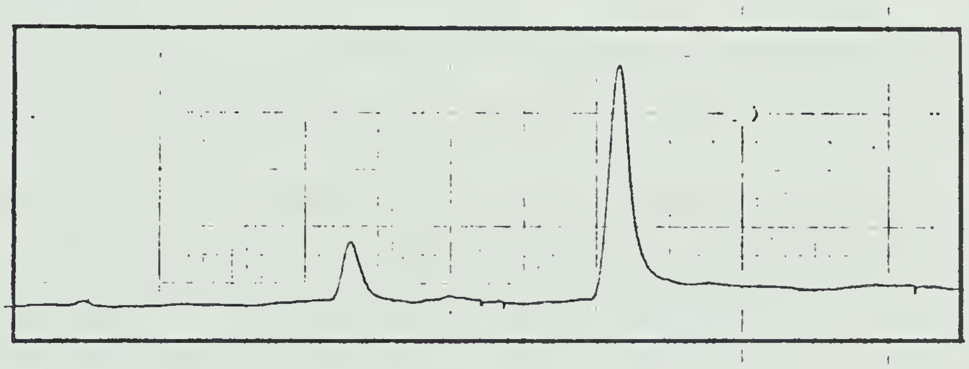


Figure 3.09 Peaks tailing from impurities in the esters obtained.

Some injections made of pure diazomethane-ether solution gave chromatograms such as those shown in Figure 3.10.

(a)



(b)

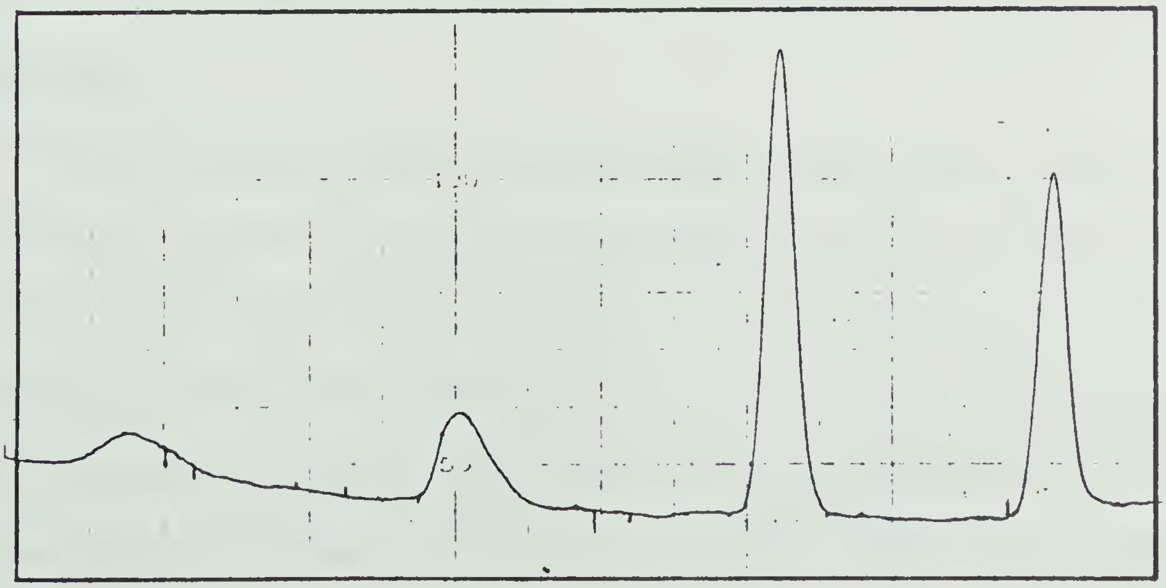


Figure 3.10 a,b Ester peaks eluted after injection of pure diazomethane through system, in a "recovery run".

The chromatograms of Fig. 3.10 were obtained by injection of diazo-ether solution immediately after the elution of the components of the previous esterification. They show the appearance of esters peaks which could have been caused by either displacement of esters left behind in some section of the reactor packing from the previous experiment or which were formed by acids retained in the same manner and esterified upon an injection of diazomethane. The yields recalculated taking into account the areas obtained for the second (or recovery) chromatograms are nearer 100%.

The possibility of polymer formation by diazomethane, giving rise to polymethylenes has been cautioned in the literature, although for longer reaction times in 'external esterifications'. Polymer formation could give rise to a series of peaks with variable retention temperatures and sizes because of the range of molecular weights possible. Apparently that is not the case here but no detailed examination of the peaks involved was attempted by means other than gas chromatography and their nature is not known with certainty.

One other pertinent observation was made concerning the reactor itself. The heated zone of the reactor starts at ~7 cm from its extremity within the injection "T". The sealing of the reactor quartz tubing in the tee is made with a Swagelok teflon ferrule and no heating of the injection section was made to avoid problems with the Teflon

ferrule and also with the septum. It is possible that in this short section the temperature was low enough to "freeze" some amount of the acids and/or esters injected/produced, giving rise to the "repeater" or "memory" effect observed when injections of diazomethane solution only were made. An effort was made to minimize this source of difficulty by wrapping a short length of heating tape around the copper tubing preceding the injection tee to pre-heat the incoming carrier gas. However some leakage developed at the Teflon ferrule and the temperature had to be lowered again.

Considering that the internal standard peak was suffering the most direct interference from the unidentified overlapping peak, one attempt was made to circumvent that difficulty by using an esterification solution containing myristic and stearic acids and whose internal standard was methyl palmitate (it elutes between the peaks of the other two esters in the chromatogram).

Response factors were calculated using data from Table 3.06, from which (A_s/A_i) for methyl palmitate were obtained:

$F_s = 1.013_4$ for methyl myristate

$F_s = 0.917_1$ for methyl stearate

The esterification mixture had the following composition:

Myristic acid, 0.2049 g; stearic acid, 0.2967 g and methyl palmitate, 0.2452 g, plus 10% by weight of methanol The solvent used was ethyl ether. Two esterification ex-

periments were run using this mixture. There was no significant improvement in the results:

Me myristate, 123%, 48.9%

Me stearate, 93.8%, 81.9%.

In view of these results, the solution was not used again and further experiments were performed reverting to the use of the previous solution using n-hexadecane as internal standard.

An examination of the chromatograms previously shown leads one to suspect that the shoulders, tailing and small peaks observed can be explained if one attributes them to small amounts of acids which were not completely esterified but were volatilized and carried into the column. Besides explaining qualitatively the appearance of the chromatograms this assumption would probably account for the discrepancies in the quantitative results.

Three experiments were run to test the above assumption: the standard solution, the esterification solution and a mixture of the two. The resultant chromatograms are shown in Figure 3.11. It is apparent that there is strong overlapping of the peaks for the acids and esters, which tends to confirm the above hypothesis. More weight is lent to this conclusion by consideration of Fig. 3.07. The absence of peaks in the region of elution of the compounds of interest seems to indicate that the diazomethane is not polymerizing under the conditions of the experiment and

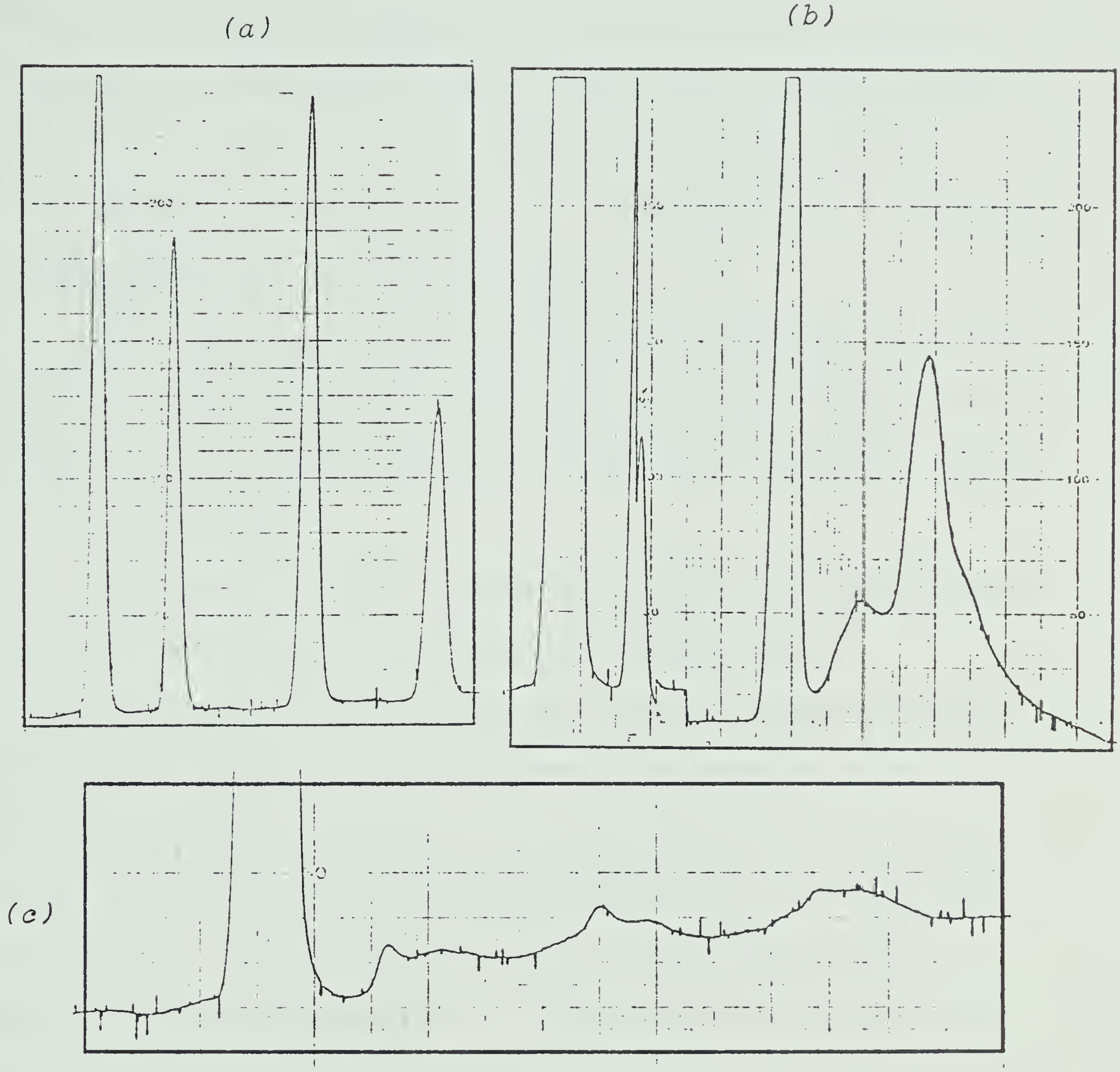


Figure 3.11 *Chromatograms of solutions for experiments with C_{14}-C_{16}-C_{18} acids (a) std. solution; (b) mixture of the two (c) esterification solution.*

thus is not introducing extraneous peaks. The excess unreacted diazomethane probably is being decomposed into nitrogen and some other product which elutes well ahead of the esters or acids. (Observations by Schlenk and Gellerman[29] indicate that in 'external esterifications' the

formation of polymers, appearance of white flocs, occurs in solutions at about 21° C when the reaction time becomes longer than 15 minutes in the presence of diazomethane).

The immediate conclusions to be drawn from the information analyzed above are the following:

1. The column is not resolving the peaks of the acids and esters. This leads to erratic quantitative data since the peaks for the non-esterified acids are integrated along with the ester peaks.

2. The esterification reaction, under the experimental conditions used, is not proceeding to completion.

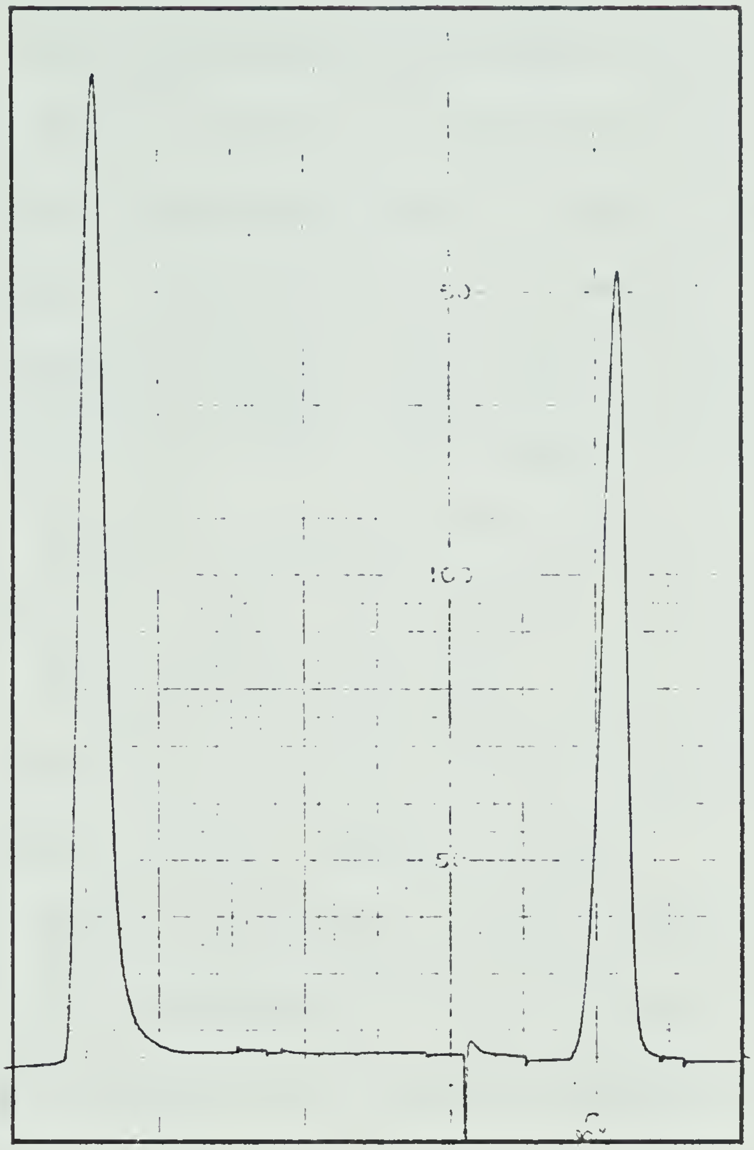
A possible explanation for the partial esterification can perhaps be given by the demands of the reagent on the one hand, and the acids to be esterified, on the other. The acids are all solids at room temperature and have high boiling points. Diazomethane tends to decompose when exposed to air at about 100° C. There is a conflict then, as to the best reaction conditions. If the esterifications are conducted with the reactor at a high enough temperature to vaporize the acids, extensive decomposition of diazomethane might occur during the residence time in the reactor. On the other hand, if a low temperature of 50° C is used, as in the experiments with octanoic acid, the higher molecular weight acids would tend to vaporize either partially or not at all and the highly volatile diazomethane would be swept away and decomposed before successful completion of

the esterification. Moreover, methanol, used in the esterification solution as a catalyst, would tend to move rapidly in the reactor, the net result being depletion of catalyst at the reaction site where the acids are located. This would contribute to reduced % conversion.

A re-examination of the esterification of octanoic acid. At this point it may be questioned if effects similar to those described immediately above were prevailing in the esterification experiments conducted for octanoic acid. The following steps were taken to obtain more information on esterification of octanoic acid.

GC evidence. Figure 3.12 shows that the octanoic acid and the methyl octanoate peaks overlap in an SE-30 column. (a) is a chromatogram of the standard solution containing ester and int. std. only. (b) is a chromatogram of a mixture of the std. solution and the esterification solution containing octanoic acid. The ester peak in (b) shows marked tailing, due to the overlap of the acid peak, whereas (a) shows the ester peak with its trailing edge returning to the base line with no tailing at all. Compared to Figure 3.02a it shows the same sharpness for the ester peak obtained during an actual esterification run, which indicates little or no contribution of the acid to the ester peak.

(a)



(b)

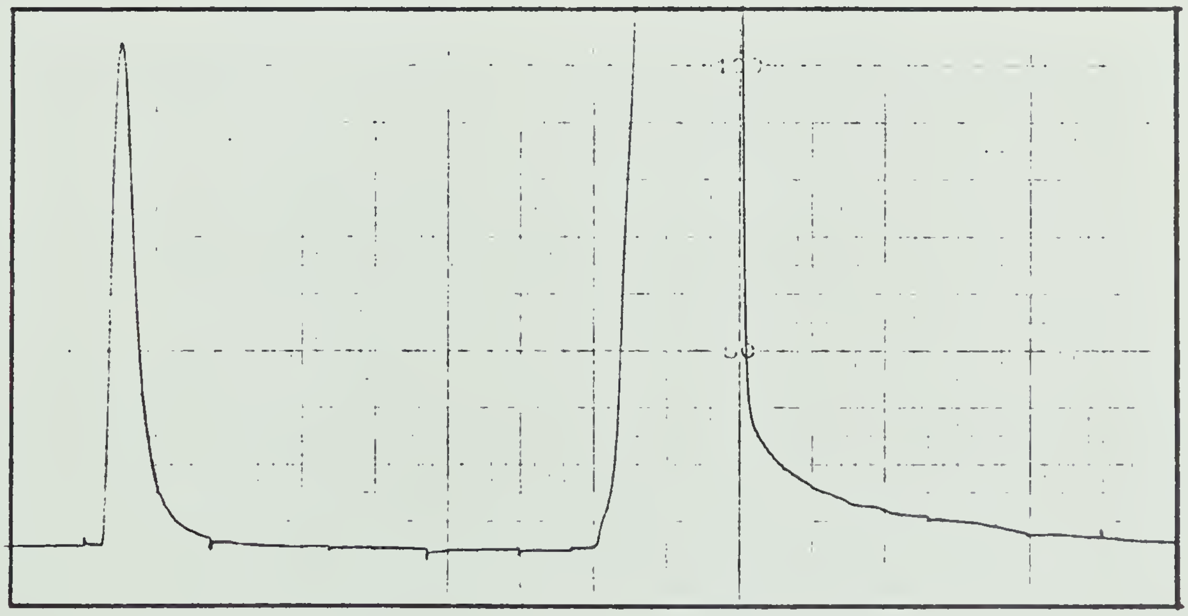
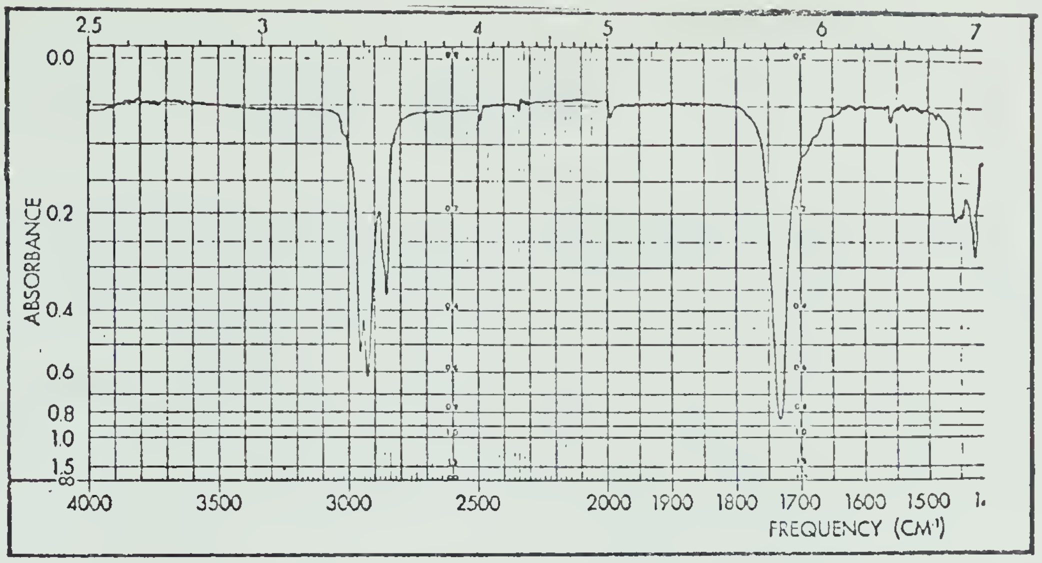


Figure 3.12 (a) chromatogram of standard solution containing, n-tetradecane (i. std), left and methyl octanoate, right.
(b) chromatogram of a mixture of std. sol. and esterification solution. Left, n-tetradecane (i. std.), right, overlapping peaks of methyl octanoate and octanoic acid.

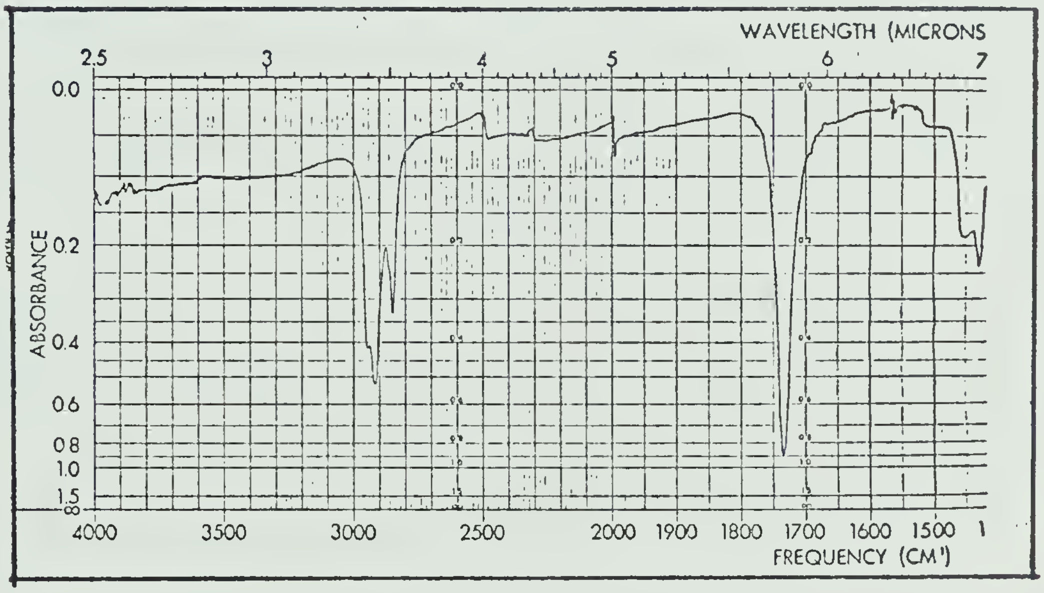
IR evidence. The chromatographic peaks were trapped using a Wilkins Corporation Model 15A GC-IR analyzer. The effluent peaks were condensed onto the KRS-5 plates of a 5153 capillary internal reflection cell by means of the thermoelectrically cooled analyzer unit and the cell taken to the Spectroscopy Laboratory[104]. The spectra obtained for the ester peak are shown in Figure 3.13, where (a) is the reference spectrum, and (b) is the spectrum of the ester peak eluted during an esterification run. The ref. spectrum was obtained from the pure ester for comparison purposes. (c) is a spectrum of pure octanoic acid.

From the spectra there is no evidence of the presence of carboxylic acid absorption in the region 3000-3500 cm^{-1} although a 10% concentration would probably be undetectable by I.R. means.

(a)



(b)



(c)

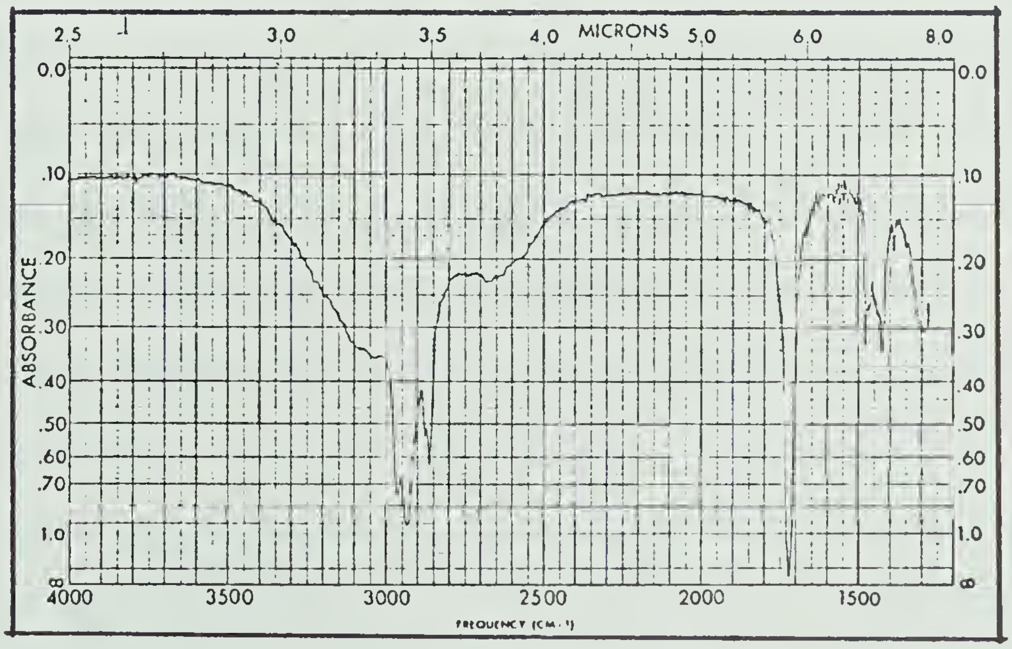


Figure 3.13 *Partial IR spectra of (a) pure methyl octanoate; (b) ester peak trapped from an esterification run; (c) pure octanoic acid.*

3.04 SUMMARY

Esterification of saturated fatty acids was examined using reaction-gas chromatography as an analytical technique. Following previous work by F. Drawert, boron trifluroide-methanol was used as a catalyst for the direct esterification through a reactor, with no previous esterification being done outside the reactor-GC system. The approach was found to produce some esterification but no quantitative evaluation was possible because of severe limitations of the method which used a calcium hydride zone for retention of acidic compounds. This proved to be difficult to prepare and cumbersome to be used in practice, besides causing interference in the elution of the compounds of interest.

Pursuing the same general method of reaction-GC, diazomethane in ether solution was employed as the esterification reagent. It was found that the method of preparation of diazomethane was critical in the attainment of high esterification yields. The common preparation using decantation from a water solution was abandoned in favor of a potentially more dangerous method, that of distillation of gaseous diazomethane into ethyl ether. No problem was encountered during the distillations conducted for this work, as far as the explosiveness of the compound is concerned. There was always the possibility however, of inhalation of the extremely poisonous reagent during syringe manipu-

lations, since all the work cannot be conducted in fume hoods. This is by far the greatest danger to the investigator working with diazomethane. This solution proved to be effective in achieving yields of 100% in the esterification of a low molecular weight acid, octanoic acid. Difficulties were encountered, however, in similar experiments conducted for the esterification of higher molecular weight acids such as myristic, palmitic and stearic for which the conditions of esterification such as optimum temperature for vapor phase reaction might result in decomposition of the reagent and incompleteness of the esterification reaction.

The effect of residence time and conversion was briefly examined but evidence was not found of a significant influence on the esterification yields. "Stopped-flow" experiments were also tried.

Polyester column partitioning phases were found to be unreliable for the present work because of interaction with the reagents employed and possible changes in their composition. On the other hand, silicone gum rubber stationary phases are not selective enough to separate esters from acids, thereby making it difficult to obtain a good material balance on the basis of the peaks for the two functional groups (acids and esters) involved.

3.05 SUGGESTIONS FOR FURTHER WORK

Several possible areas for further work can be surmised from the results and conclusions reached in the previous sections.

Although the use of commercially available GC instruments are often frowned upon by many researchers, an instrument such as the Varian Aerograph 2100 gas chromatograph or a similar instrument would be a valuable machine with which to do reaction-GC research especially on problems related to biochemistry and life sciences. Its built-in capabilities would provide flexibility for the separation-detection section of the chromatographic experiment allowing the investigator a free hand to concentrate on the many problems brought about by the coupling of a reactor to a gas chromatograph. In the research described in the present work it was seen that many problems which arose during its realization were due to instrument design only and much time was lost on the modification and improvement of the 'home made' GC system. Although such an approach can be of didatic value in acquiring some experience in the arrangement of separate components of a gas chromatograph and in making them work satisfactorily it is my personal opinion that it is difficult to compete with the skilled engineering of commercial firms which manufacture good equipment. Their equipment when judiciously chosen with a definite aim in mind will lend itself

to minor adjustments and changes destined to better adapt them to the specific problem at hand.

Once the problem of a suitable GC instrument has been decided upon, the study of variations in reactor design could be taken up. It would be interesting to have a system in which two distinct flows were possible, one in the reactor itself, the other in the GC instrument. This would allow variation of the flow within the reactor without disturbance of the column flow for a more detailed investigation of the effects of residence time on the esterification. It might also provide an answer for the problem encountered with acids of higher molecular weight, by placing the mixture to be esterified in some reacting chamber where the acids and reagents could be well mixed and make good contact before raising the temperature and changing the flow pattern to begin the transportation of the reaction products to the separation column. While the reactor used in the present work has characteristics of simplicity in construction,use and maintenance,it is perhaps oversimplified for the task to be performed and experimentation with other designs cannot be overemphasized.

As to the esterification reagent itself some investigation should be made of diazoethane or diazobutane for esterifications by reaction-GC on their stability, lack of selectivity and toxicity among other factors which might indicate a possible alternative for diazomethane.

The main task to be faced will be that of investigating the esterification capabilities for samples covering a wide range of molecular weights of fatty acids, both saturated and unsaturated, in order to affirm the applicability of Reaction-GC as an analytical tool for this specific problem.

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APPENDIX I

SECTION 1 EQUIPMENT

Basic apparatus

Owing to the fact that no integrated commercial type of GC instrument was available in the laboratory, some time was spent in putting together a system for use in the present investigation. In this Section a somewhat detailed account of the construction and modifications made on equipment is given.

A. Column heating

The first approach used for a column heating system was employing a dewar flask as a reservoir for an oil bath. The flask was 22 cm in diameter x 30 cm deep. Two Cenco 450-watt knife heaters were immersed in the oil which was a 335/350 SSU viscosity mineral oil. As a preliminary test, the bath temperature was raised to 100° C the detector at 250° C and the injector at 290° C. The column used was a 2-meter ⅛" O.D. stainless steel tubing packed with SE-52 phenyl silicone rubber phase on Chromosorb W HMDS-treated (80-100 mesh) at a loading of 8%. The temperature of the oil bath had to be raised in order to elute C_8 acid in a reasonable time but the oil soon began to vaporize and fume badly. It was substituted for an SAE 30 motor oil, later

an SAE 90 pure mineral oil was tried but there was evidence of oxidation and decomposition and the idea of using an oil bath had to be abandoned, since it would be impossible to use it in the range of temperatures needed.

After some time an oven used on an F & M 500 programmed temperature GC instrument (Figure A.I.1) was obtained from the electronics shop and adapted to the Gow-Mac TC cell box by means of a lateral platform. Connections were made to

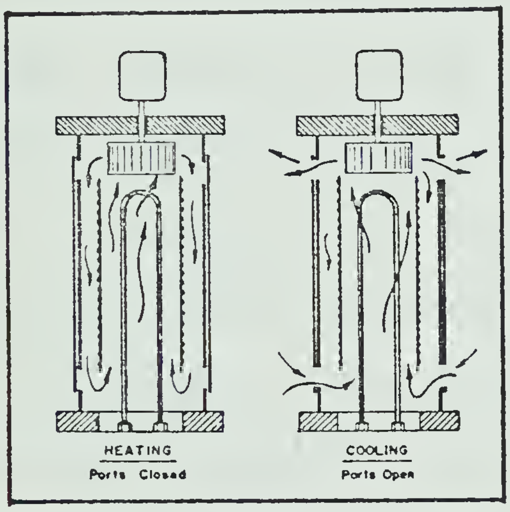

Figure A.I.1

Vertical cross-section of F & M Model 500 linear programmed GC oven showing air circulation

the cell by means of ⅛" O.D. stainless stell tubing, although internally all connections to the cell block are made with copper tubing having an O.D. of 3/16". The oven was placed on the platform on top of a fiberglass mat 0.5" thick which behaved well as a heat insulator. This arrangement was awkward because it left the connections exposed to the atmosphere and at a lower temperature than in the oven or in the detector box. To remedy this the lines were wrapped with heating tape. It was later realized that this setup was deficient because of the poor temperature control pro-

vided by the heating tapes. In a drastic modification the whole setup was altered as described under "detector", below.

The F & M oven was kept for the duration of the present work. One criticism to be made of this oven is its small inside diameter which leaves no alternative but the use of columns coiled to a maximum diameter of 3 inches, contrary to recommendations of the literature for best resolution. The only modification made on the oven during the period of time it was in use was changing its original fan motor to a higher speed lawn mower motor to eliminate some of the temperature gradients due to slow circulation of the air. The motor, however, was noisy and vibrated too much. It was finally substituted for a stirrer motor with speed control, having nominal shaft speeds up to 5000 r.p.m. The manufacturer is Gerald Heller, Co., which also supplies a Thyratron controlled speed limiter, GT-21, which is also a rectifier unit.

B. Temperature programmer

An Aerograph model 326 Linear Temperature Programmer was used. All the GC work done was under programmed temperature conditions. The programmer was connected to the F & M oven and the combination proved to be a satisfactory one.

Initially a graph of initial temperature dial setting vs. oven temperature was plotted for future reference. The graph is seen in Fig. A.I.2.

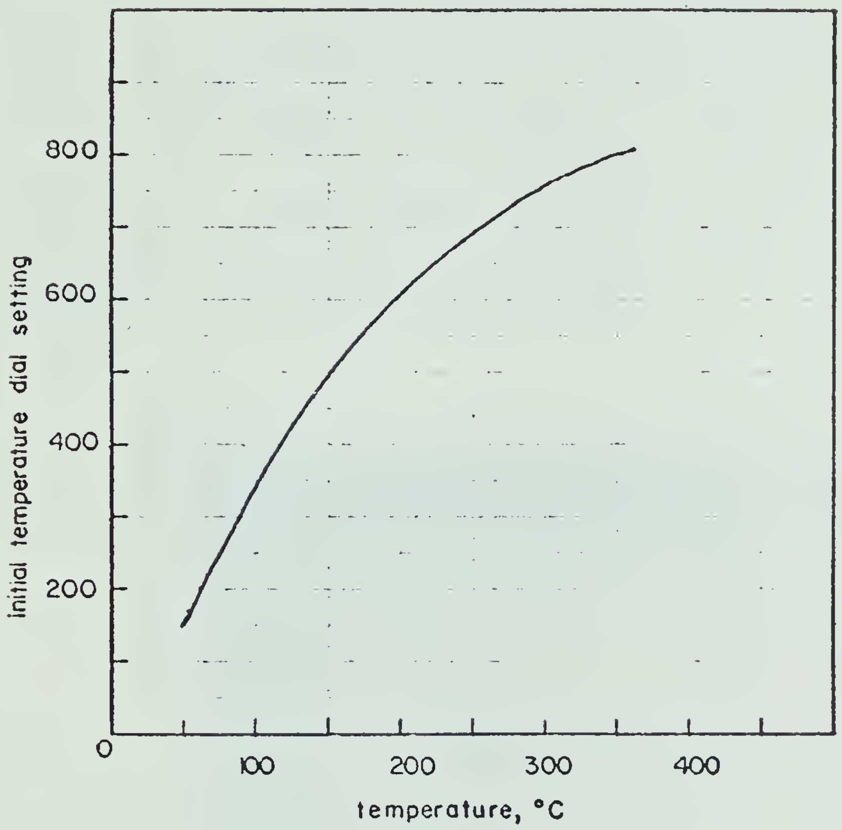

Figure A.I.2

Calibration graph for Aerograph 326 Linear Temperature Programmer

C. Detector

The detector used was a thermal conductivity cell, Gow-Mac model TR-II-B, in conjunction with its associated power supply, 9999-C. The filament current was set at 200 mA and the detector block was maintained at 250° C at first by means of the cell's own internal thermostat.

Modifications

1. The first modification made on the TC cell was to change the inlet tubing to the sample and reference sides for stainless steel. In the original arrangement the copper tubing from sample inlet was a long one and passed below the detector block through the thermostat block and

then back in to the detector, as seen in Figure A.I.3. That not only increased the dead volume of the inlet tubing but also increased the time of sample contact with hot copper tubing. A modified version of the TC cell was designed to improve these shortcomings.

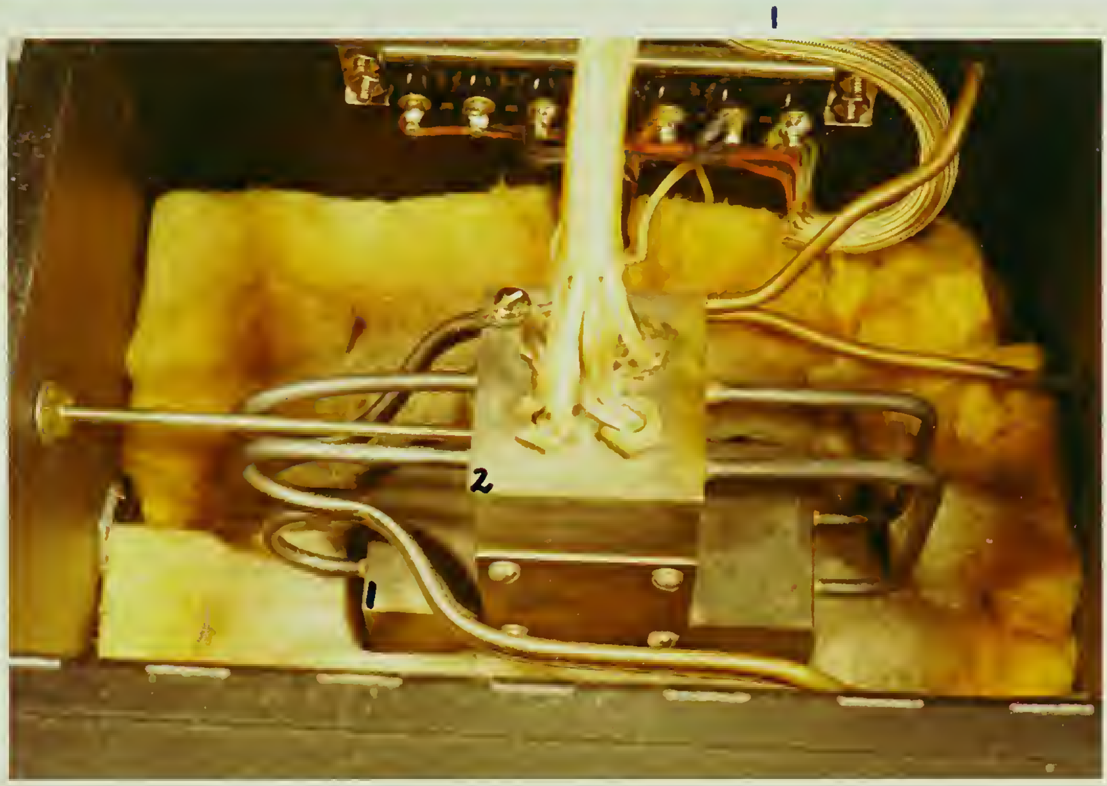

Figure A.I.3 Interior of a Gow-Mac Model TR 2B thermal conductivity cell showing arrangement of tubing. 1 - heater block; 2 - detector block.

Short lengths of copper braid, 0.5" wide were wrapped on the remaining stub of copper tubing still attached to the thermostat and then onto the new stainless steel inlets, so that heat could flow among them and keep the inlets at a high temperature. The original glass wool insulation was replaced.

2. Another modification was made much later. It was found that the lines connecting the TC cell to the column in the lateral platform were overheated by the non-uniformity of the heating obtained from the tapes used. To improve on this situation an opening of about 2 cm diameter was opened on the side panel of the TC cell opposite its electrical connections panel and a short length of tubing was prepared using a $^3/_8$" Swagelok reducing union modified by welding to it a piece of $^1/_4$" OD 316 stainless steel tubing. This became the connecting 'bridge' between the reactor and the column inlet end. The tubing connecting the outlet end of the column to the detector block was taken out of its normal position on the side of the box and so positioned that the column outlet was connected directly to it. The other extremities of both pieces of tubing were bent to a vertical position, pointing up towards two openings on an aluminum plate which replaced the original hinged top of the TC cell.

To avoid condensation of sample in the two pieces of bridge tubing, two Hotwatt 50-watt 115-volt pencil type cartridges were placed close to the bridges in such a way that one of them was parallel, in a horizontal position, to the inlet from the reactor; and the other also roughly parallel, in a vertical position, to the inlet from the column to the TC cell. Two thermocouples were insulated with a thin Teflon film and attached to the outside walls of the bridge tubing to provide temperature readouts for

them. The heaters were connected in parallel to a Powerstat transformer. Figure A.I.4 shows the final version.

Figure A.I.4 Modifications introduced into the T.C. cell. 1 - column; 2 - reactor outlet; 3 - column outlet; 4, 5 - additional heaters; 6 - detector block.

The last modification made was to connect the TC cell heater to an API proportional temperature controller so as to minimize baseline drift due to cycling of the cell's thermostat which was observed previously.

D. Recorder

The recorder used was a Sargent Model SR strip chart recorder with a 1.0 second pen response time, 1.0 mV full

scale range to which a stepped attenuator had been adapted. Its chart speeds were 0.2, 1.0 and 5.0 inches per minute.

E. Integrator

An Aerograph model 471-42 digital integrator was used for the quantitative determinations made. The recorder chart provided mainly a visual 'read-out' in the form of the usual chromatographic peaks but the integrator was used exclusively for obtaining quantitative digital data from sample peaks.

F. Temperature read-outs

Those were made using two 5- and 6-thermocouple stations prepared in the Electronics Shop of the Department of Chemistry. The first box had its six points located in the following way:

#1: TC cell block;

#2: Bridge from column outlet to detector;

#3: Bridge from reactor to column inlet;

#4 and #5: Column oven;

#6: Temporarily, reactor.

The second box:

#1 and #2: Also connected to column oven;

#3, 4, 5: placed in contact with the reactor insulation.

G. Gas flow indicators and controllers

A Matheson model 8 (CGA) two-stage regulator with a delivery pressure range of 4-50 psig was adapted to the helium

cylinders.

Two flow controllers were used: one of them was a Brooks ELF model 8744 designed for constant upstream, variable downstream pressure. The following specifications were taken from a design specification DS-8743-8744, Feb. 1969, Sec. J, furnished by the manufacturers, Brooks Instrument Division of Emerson Electric Co., Penn. U.S.A.:

- NRS (non-rising stem) valve
- 316 SS body and needle valve, rated at 300 psig
- Kel-F "O" rings
- Teflon controller diaphragm
- Capacity: #1 Taper needle, 750 scc/min.
- Control precision, for any flow rate between 2 and 200 cc/min, the rate will not change more than 0.3% of the instantaneous flow rate when operating with helium at 50 psig and a downstream pressure variation from 0-40 psig.

The other flow controller was a Millaflow constant upstream controller, Model PN-42300080, with extra-fine metering valve. It was first adapted to the column side, later transferred to the reference side of the detector. Its general specifications are:

- Brass body. Maximum inlet pressure, 150 psig
- Flow range: approx. 90 ml/min at 20 psig to approx. 400 ml/min at 150 psig.
- Flow repeatability: flow is stable and repeatable

within ±2% of flow value under the following conditions: 1) the reference pressure (upstream or downstream) varies no more than ±1%; 2) the difference between inlet and outlet pressures is at least 10 psi; 3) ambient temperature varies no more than 10° F.

The manufacturer for this type is Veriflo Corp., Richmond, Calif., U.S.A., Millaflow Division.

To better visualize the flow a Matheson 150 mm four tube flow meter unit was installed between the carrier gas cylinder and the flow controllers. The unit was not calibrated to provide direct reading of flow because all critical flow measurements were carried out using a soap bubble flowmeter.

H. Gas purifying cartridge

An indicating molecular sieve trap containing "Hylar M" 13 X molecular sieve and activated charcoal, obtained from SKC Inc., Pittsburgh, Pa., U.S.A., was used to remove impurities such as water and trace hydrocarbons from the carrier gas.

SECTION 2 CHEMICALS AND GC MATERIALS

A. Solid Supports

The solid supports used for the preparation of 1/8" OD

columns was Chromosorb W, HMDS treated, in the 80/100 mesh range. For the preparation of ¼" OD columns, Chromosorb W AW, 60/80 mesh was used. Both mesh sizes are in accordance with recommendations found in the literature for the diameter of the columns used. Both types of solid support are manufactured by Johns-Manville, U.S.A.

B. Stationary phases

SE-30 and SE-52 silicone rubbers were obtained from Chromatographic Specialties, Brockville, Ontario, and F & M Scientific Corporation.

Porapak types Q- and Q-S were used, obtained from Waters, Associates, Inc., Mass., U.S.A.

Neopentylglycol succinate (NPGS) was also used for some time. It was also obtained from Chromatographic Specialties.

C. Column tubing

Two sizes were used, ⅛" OD and ¼" OD, 316 Stainless steel, manufactured by Superior Tube Co., Pa., U.S.A. The tubing was washed following the recommendations of Teranishi[93].

D. Syringes

The following types of syringes were used:

1. For small sample injection.

   Hamilton 7001 N, 1 microliter capacity

Hamilton 701, 10 microliters capacity, adapted with a Kel-F guide

Hamilton 705, 50 microliters capacity

Precision Sampling Pressure Lok Series CG, liquid syringe, 10 microliters

Precision Sampling, idem, Series A, gas syringes, 100 microliters.

2. For preparative work, a Hamilton Gas-Tight #1750 syringe was employed, with a capacity of 500 microliters.

The Hamilton 7001 N syringe was found to have poor repeatability. The 701 was acceptable but its stainless steel plunger eventually became etched by repeated injections of BF_3-methanol reagent in spite of frequent washing with dry methanol following every injection. The Precision Sampling Pressure-Lok gas syringes were found to be good for general sample injection as well as use with ethereal diazomethane solutions because of their specially designed valve. Leakage was practically non-existent and this made it possible to inject samples with improved repeatability over the Hamilton syringes.

E. Chemicals

1. Specifically used for boron-trifluoride-methanol reactions:

Gaseous BF_3, lecture bottle, Matheson of Canada, Ltd.

Methanol (Spectrograde), Eastman S 467, Lot No. 629 E

Calcium hydride, BDH, 85-90% CaH_2

Calcium hydride, Ventron Corp., Metal Hydrides Division, Lot J-4169 A, 4/40 mesh.

2. Specifically used for Diazomethane esterifications:

N-Methyl-N'-Nitro-N-nitrosoguanidine, Aldrich Chemical Co., Inc., Wisconsin, U.S.A.

Ethyl ether, anhydrous, Mallinkrodt 0848, analytical reagent

Benzene, Fisher Spectranalyzed, Lot 785133

Benzene, Eastman Spectrograde, Lot 681A

Normal tetradecane, normal-undecane, Pure grade, 99 Mol % Min., Phillips Petroleum Co., Oklahoma, U.S.A.

n-Hexadecane, Eastman P3388, Practical Grade

2-(2-ethoxyethoxy)ethanol, ethyleneglycol monoethyl ether, Practical, Matheson, Col. & Bell.

3. For esterifications by both methods:

Propionic acid, Fisher Certified Reagent, A-258

Methyl propionate, Eastman 746, Red Label

n-Butyric acid, Eastman 746, Red Label

Methyl n-butyrate, Eastman 787, Red Label

Octanoic acid, Eastman 665, Red Label

Methyl octanoate, Eastman 891, Red Label

Octanoic acid, methyl octanoate, myristic acid, methyl myristate, palmitic acid, methyl palmitate, stearic acid, methyl stearate, obtained from Mann Research Labs., Inc., New York, Assay >99% by TLC.

F. <u>General purpose: TLC analyses, titrations, etc.</u>

Benzoic acid, Fisher PA grade

KOH, BDH Laboratory reagent

Potassium hydrogen phthalate, Matheson Coleman & Bell, alkalimetric standard.

Hexanes, Fisher ACS certified (mixed isomers), Lot 780333.

Chloroform, Analytical reagent grade, Mallinkrodt 4440.

Iodine, I-37, Fisher Certified, ACS., resublimed, Lot 710810

Aluminium Oxide G, No. 08077, with gypsum binder for TLC, American Optical Corporation, Richmond, California, U.S.A.

APPENDIX II REACTOR DESIGN

1. First model

In the initial attempts at a practical reactor design, the following desirable characteristics were kept in mind:

The injection port section of the reactor should be built so as to minimize or eliminate sample flash back.

The reactor proper should be easy to bring to a desired temperature and to assemble or dismantle.

Both sections should have independent means of heating.

Figure A.II.1 shows the first model built. The injection

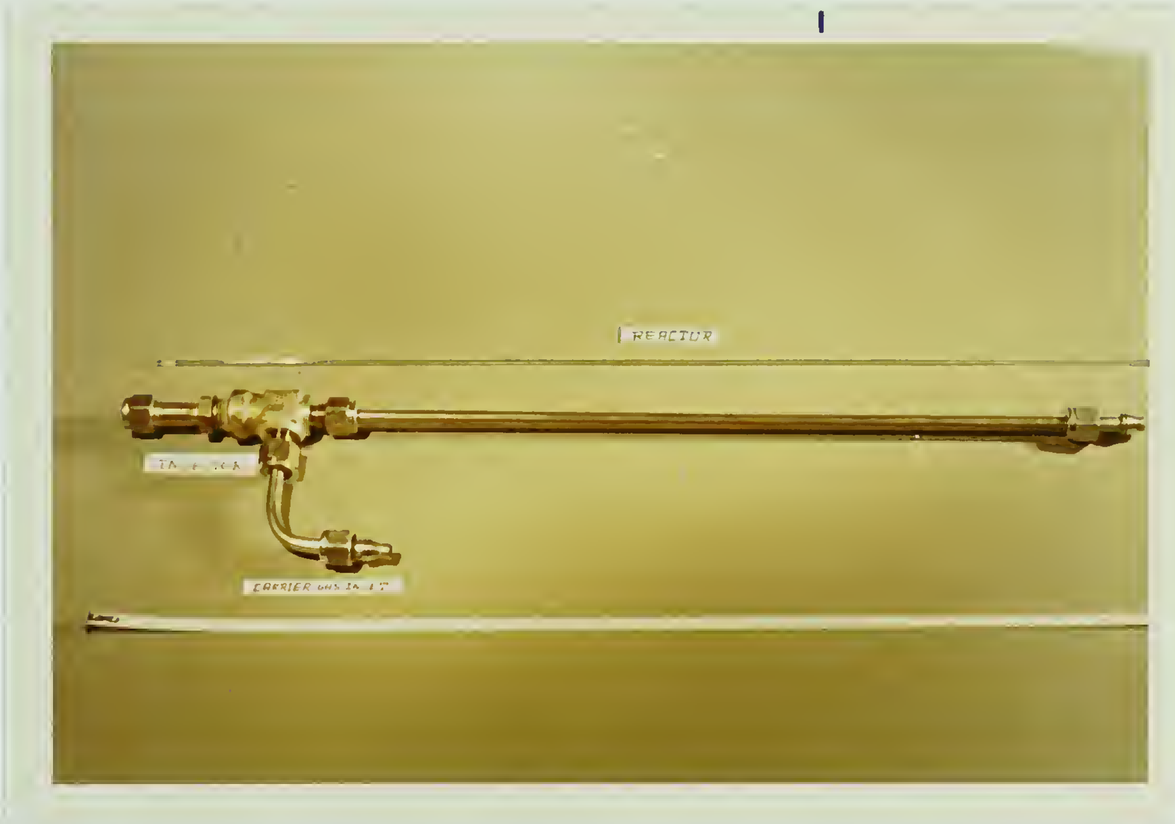

Figure A.II.1 First reactor, with its metal sheath

section was composed of a Swagelok female run tee (Cat. No.

400-3-4TFT) with a 1/4" T tube OD and 1/4" P female pipe size, to which was welded a Swagelok bulkhead male connector (Cat. No. 400-11-4). The 1/4" tube end of the bulkhead connector was used as a septum retainer, its sharp internal rim machined down to a 1 mm flat lip for better accommodation of the septum. An ordinary 1/4" Swagelok nut fitted with an inverted back ferrule was the actual septum retainer. The 1/4" tube end of the female run tee was used as the attachment point for a 41.8 cm length of 1/4" S.S. tubing which served as a mechanical support for the glass tubing reactor; the lower connector on the tee was used as the carrier gas inlet. Since the annular space between the stainless tubing and the walls of the injection section was narrow (less than 1 mm) it is believed that the high velocities attained by the carrier gas were sufficient to prevent sample flash back. The opposite end of the stainless tubing was provided with a Viton rubber O-ring used to seal off the reactor from it and was attached to the column inlet by means of a reducing union from 1/4" to 1/8". The reactor tubing was a thin-walled Pyrex glass tubing of approximately 2 mm I.D.

Heating was accomplished by heating tapes wound on the injection and reactor sections separately and controlled by variable transformers (Powerstats). Some thermocouples were positioned under the heating tapes in contact with the stainless steel sleeving and one of them was inserted in a short well drilled on one side of the female run tee. Later, an

approximate 'temperature profile' was obtained by inserting one thermocouple directly inside the glass reactor through the open injection port. The graph obtained, shown in Figure A.II.2 shows clearly that the temperature distribution was far from uniform and that it was difficult to obtain a better performance from heating tapes.

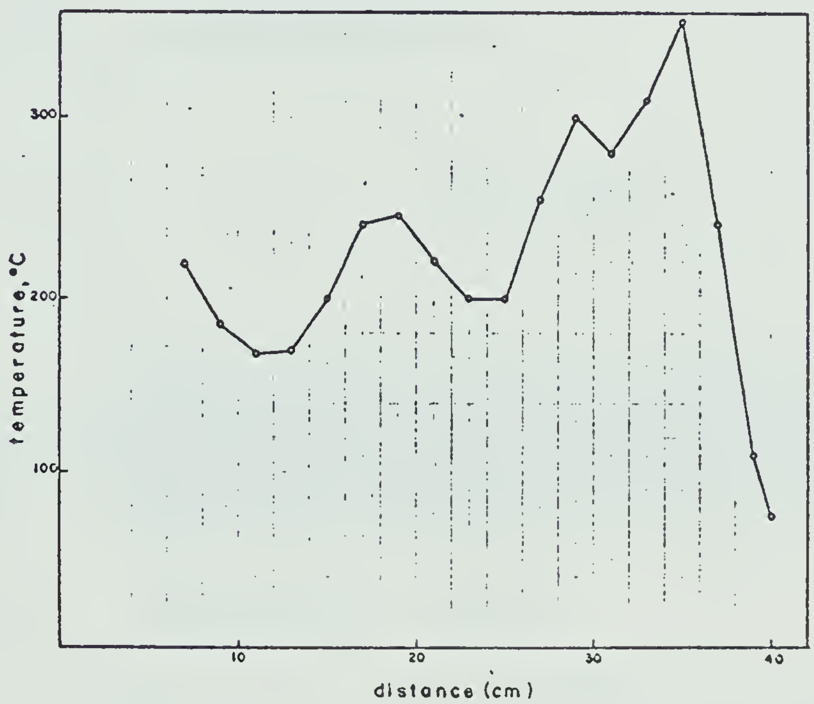

Figure A.II.2 Temperature profile obtained for the reactor shown in Figure A.II.1

2. Metal block design

It became apparent, from the experiments made with the first model described, that for uniform heating one would have to resort to a metal block of large heat capacity

instead of using the arrangement with heating tapes which have the undesirable characteristic of producing 'hot spots' because of uneven stretching over the area to be heated.

Although there are several descriptions of reactors in the literature, as was seen in Section 1.09 of Chapter 1, the design proposed by Andréu[103] seemed to be adequate for our purposes. Figure A.II.3 depicts its essential parts as well as a graph of its temperature profile at two operating temperatures, as given by Andréu.

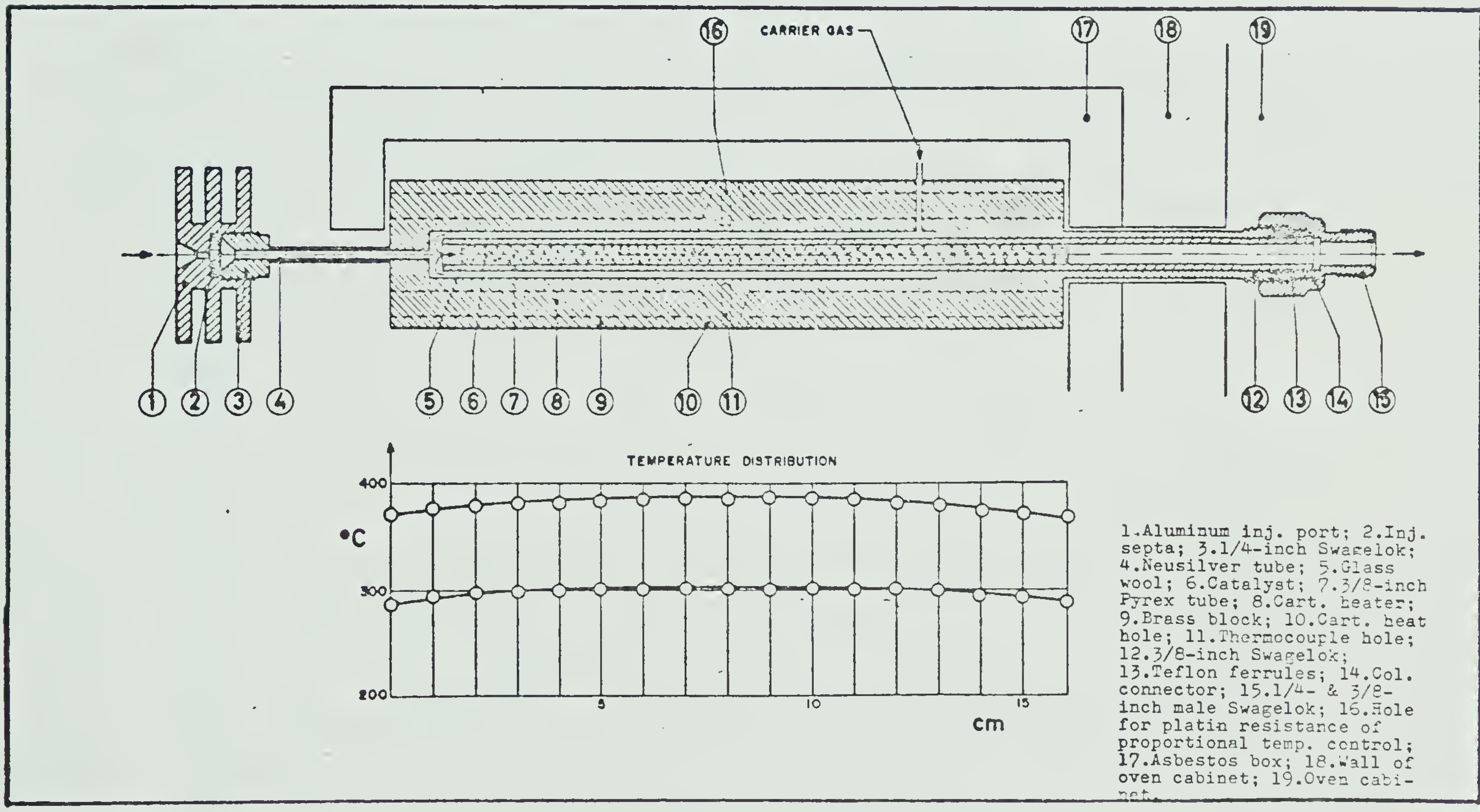

Figure A.II.3 Reactor according to Andréu[103]

The most attractive feature of the reactor shown is the

possibility of using Pyrex or quartz tubing inside the brass block. For use in this work some modifications were introduced in the basic design. The brass block was electrically insulated and chromel resistance ribbon wound on it evenly and closely spaced. One layer of Fiberfray 970 J (Carborundum Co., Penn., U.S.A.) was placed on top of the heater followed by a layer of aluminized fiberglass (Scotch Shield-3 M Co., U.S.A.) as a cover material. The thermostating and control of the reactor was done by an API model 226 proportional temperature controller with a thermocouple inserted on one side of the brass block as a temperature transducer, another located diametrically opposite as a temperature read-out. Figure A.II.4 shows a temperature profile of the reactor as obtained by use of a Hewlett-Packard model 2801 A quartz

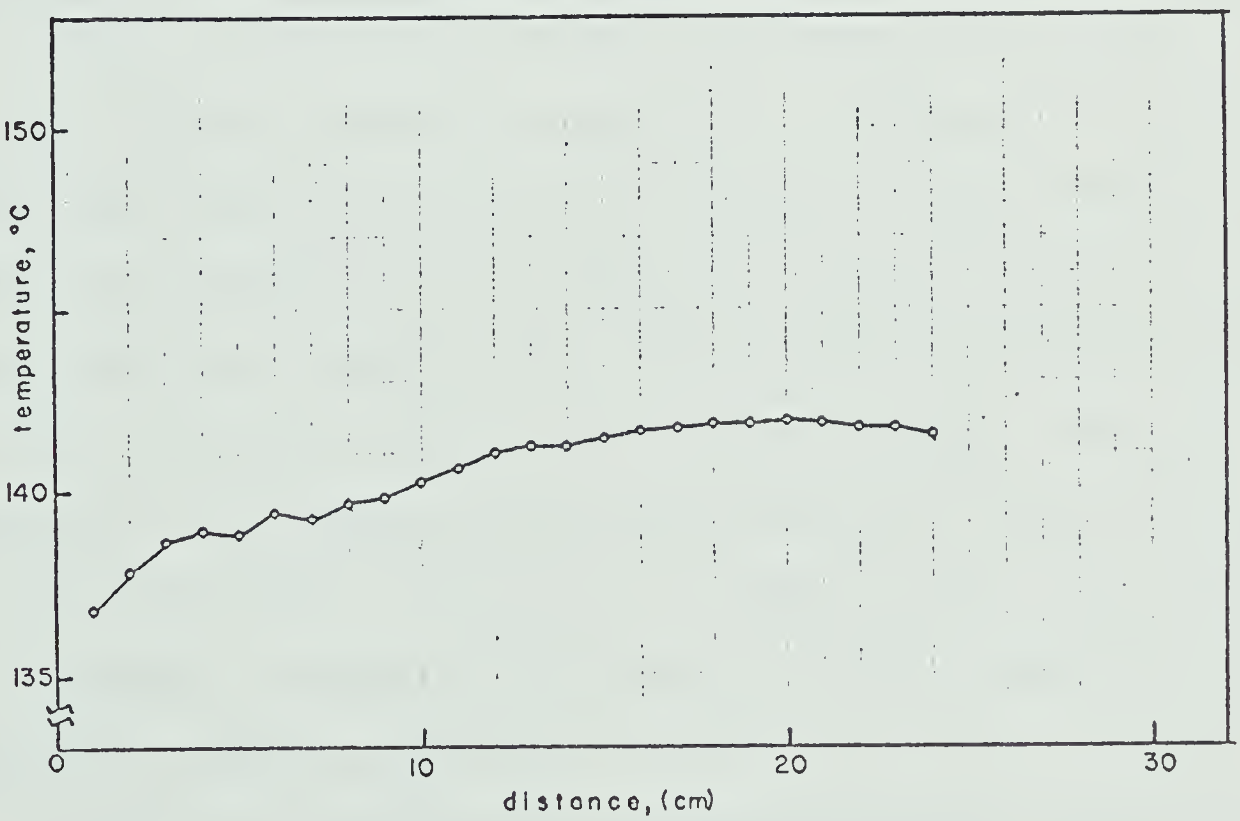

Figure A.II.4 Temperature profile obtained for a reactor similar to that shown in Figure A.II.3

thermometer. It shows some fall off at one of the extremities where the rear brass tubing was conducting heat much faster than the injection port tubing made of stainless steel. In actual use, however, these portions were not exposed but insulated so that heat loss was less significant.

Experience showed that the metal block was effective in maintaining the temperature inside the reactor at a preset value with the help of the proportional controller. Later, however, its large mass became a hindrance when it was desired to lower the temperature rapidly. Raising the temperature was not too slow because of the large current input of the API controller to the heater coil in the initial stages of the heating cycle but still it was not fast enough in some occasions where time was of importance. Another problem was the number of Swagelok fittings which had to be used to connect the reactor to the column inlet. This proved to be cumbersome when dismantling the reactor, whose internal glass tubing was accessible only through the back orifice. At this stage the glass inserts were Pyrex capillary tubing of 9 mm OD, 1.5-2.0 mm ID and 28.5 cm in total length. The extremity of the tubing facing the septum was flared into a conical receptacle to serve as a needle guide; the other end was slightly enlarged to 9.5 mm so that the sealing O-ring would fit tightly onto it.

In a later modification, shown in Figure A.II.5, the brass body was completely bored out through the septum end.

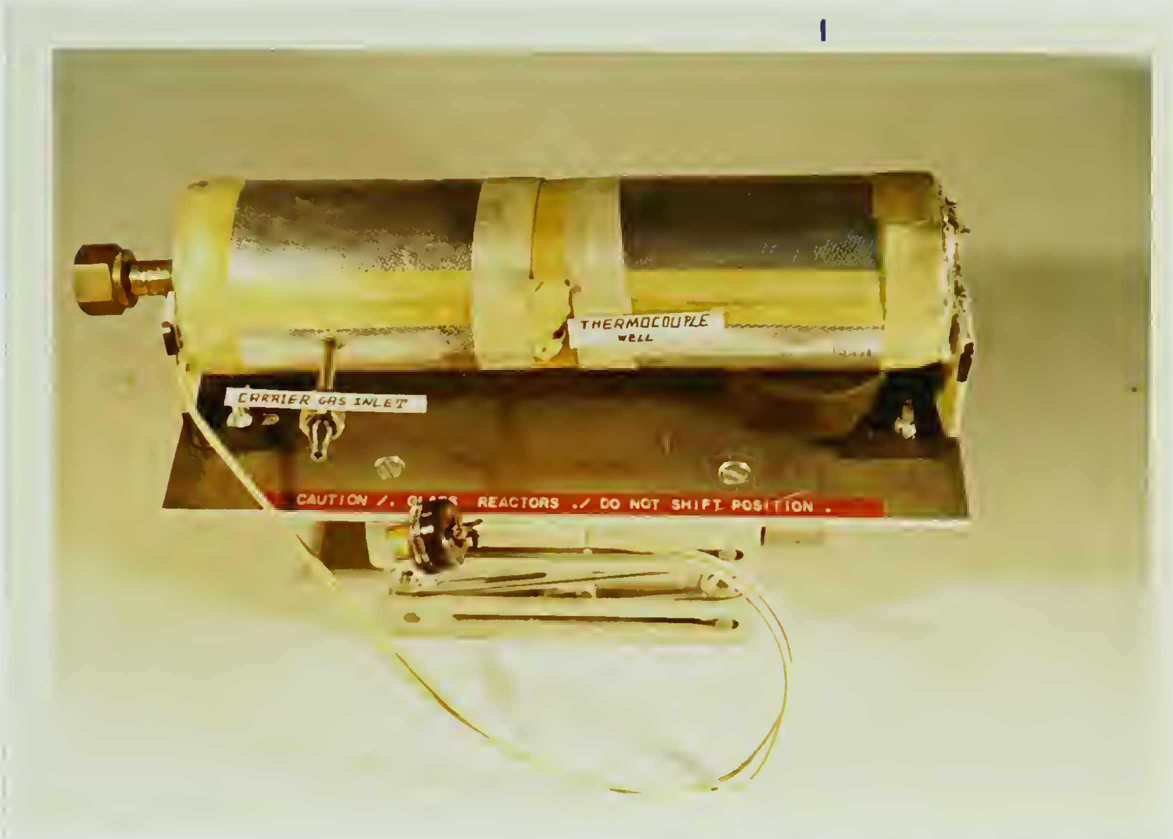

Figure A.II.5 Brass block heater for reactor after removal of injection port extension. Also shown is the Pyrex reactor

Now, the glass or quartz reactors would pass through the brass body and at the front end a Swagelok $^3/_8$" tee was connected to serve as an injection port. The other end was not modified and connected the reactor to the column inlet in the usual way.

3. <u>Final version</u>

The final version of the reactor is shown in Figure A.II.6. It was the simplest solution to the problem of fast heating and cooling cycles. The reactor tube was a quartz tubing of 7 mm I.D., 38 cm long, approximately 26 cm

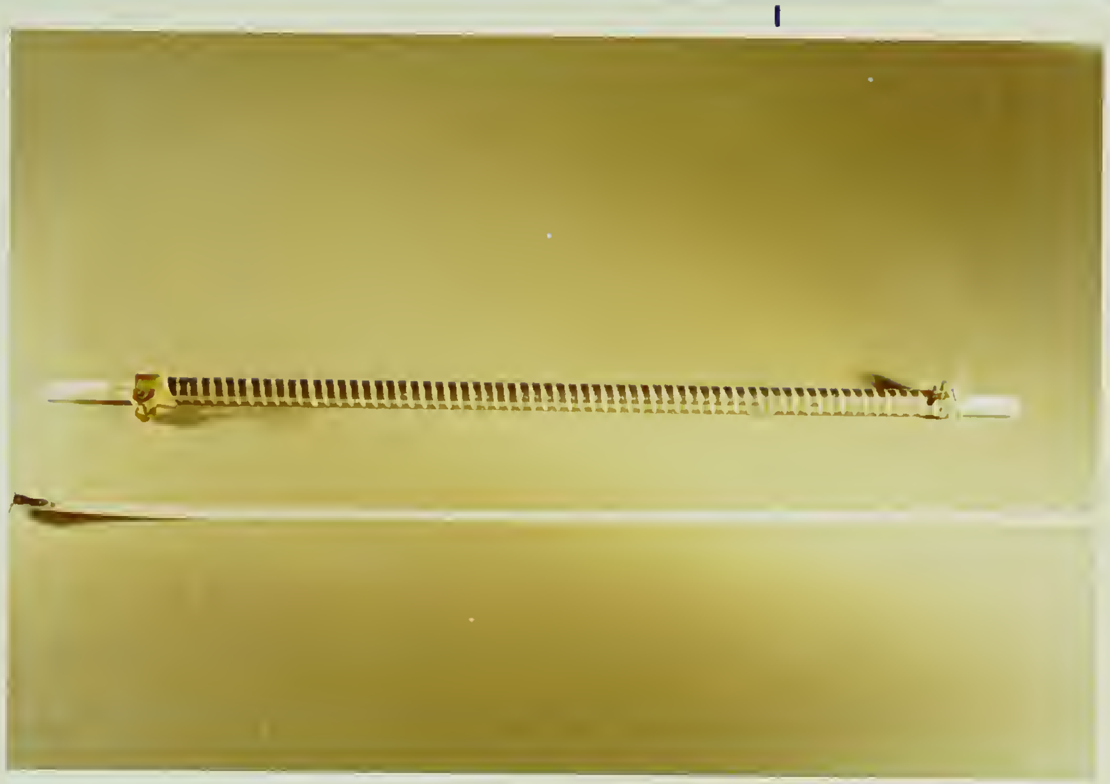

Figure A.II.6 Final version of the reactor, showing the quartz tubing covered by a sheath of fiberglass cloth and the arrangement of the heating band

of which was covered by a fiberglass thermal-electrical insulating sleeving. On this sleeving a chromel resistor ribbon was wound as evenly spaced as possible. Two electrical cord wires were silver-soldered on brass clamps used to maintain the resistor windings in place, electrical connection being made to a variable transformer. A length of 3 cm O.D. quartz tubing was used as an outer insulating sleeve, resting on two Transite spacers. A layer of Fiberfray insulation covered by aluminized fiberglass is optionally slid over the quartz sleeve to serve as further insulation but it increased

the cooling time. The temperature may be monitored by inserting up to three thermocouples between the quartz sleeve and the reactor, through small openings on the sides of the Transite spacers.

APPENDIX III

CONFIGURATIONS OF REACTOR-DETECTOR-COLUMN OVEN

In Chapter 1, Figure 1.08 depicts the basic units which were to compose the system for the research to be conducted. During different phases of the work, however, various configurations of the triad: oven-reactor-detector were tried until the most satisfactory one was found which remained in use for the remainder of the investigation. They will be briefly described here in chronological order.

Configuration "A"

This was the first one to be used and was laid out based on the reactor design shown in Figure A.II.1. The oven was placed on a platform attached to one of the vertical panels of the TC cell box and connections to the column from the reactor, and to the detector from the column, were made with ⅛" O.D. stainless steel tubing. Heating of these external lines was accomplished by wrapping fiberglass heating tapes around them and raising the temperature by means of a Variac.

Configuration "B"

The geometry remained the same as in "A" the changes being the following. (a) the brass block with glass reactor insert was substituted for the previous reactor shown in

Figure A.II.5. The temperature of the block was controlled by an API proportional temperature controller. (b) a Republic 3-way valve was used to provide an independent injection port for samples when it was desirable to bypass the reactor.

Configuration "C"

In this assembly, the brass block heating system was abandoned and the reactor is the one described in Appendix II.3 and shown in Figure A.III.1.

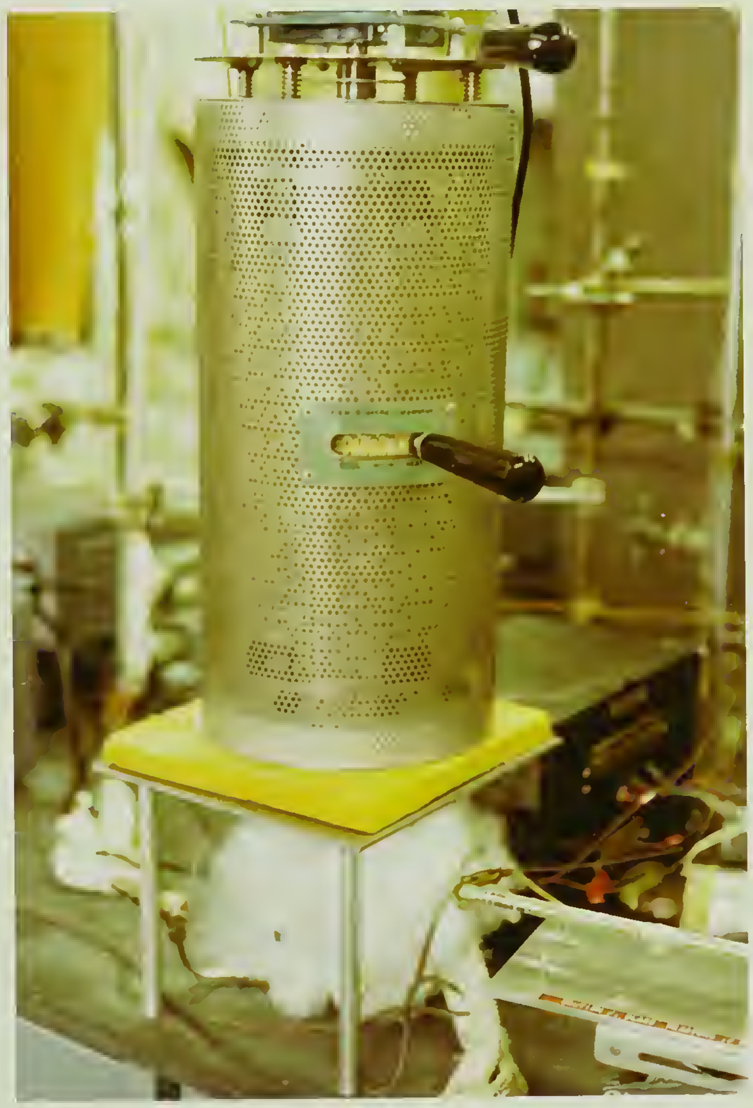

Figure A.III.1 Configuration "A", showing placement of F & M oven, TC cell in the background.

The geometry of positioning of oven and TC cell remains the same as in the previous layouts. The heating is still done the same way. A small modification was introduced later by insulating the space underneath the platform with glass wool to have a more uniform distribution of heat on the connecting lines.

Configuration "D"

This was a complete modification of the reactor-detector configuration. The reactor design remained the same as in "C" but the oven platform was removed, the oven now resting on a glass fiber mat placed on top of the cover of the TC cell box and the connecting lines are those described in section 1.C, Appendix I, for the modifications made internally on the TC cell.

A slight improvement on this design was later made by placing the Swagelok fitting for the quartz reactor completely inside the TC cell box, thus avoiding a "cold spot" which was present in its previous location. The reactor was also protected against drafts and variations in temperature by a jacket consisting of two halves of a 30 mm quartz tubing held in place by two Transite spacers. This last configuration, considered the most satisfactory and flexible one, can be seen in Figure A.III.2.

Figure A.III.2 Configuration "D", final